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The Journal of Allergy

VOL. 6 MARCH, 1935 NO. 3

Original Articles

STUDIES IN MUCOUS MEMBRANE HYPERSENSITIVENESS*†

I. PASSIVE LOCAL SENSITIZATION OF THE OPHTHALMIC MUCOUS MEMBRANE

MATTHEW WALZER, M.D., HYMAN SHERMAN, M.D., AND
LOUIS A. FELDMAN, M.D.
BROOKLYN, N. Y.

IN MOST of the investigations into the nature of atopic hypersensitiveness, the skin has been utilized as the experimental shock tissue. Cutaneous testing with allergens and specific passive local sensitization of normal skins with the blood serum of sensitive patients (Prausnitz-Küstner phenomenon) are the procedures which have yielded most of the available information concerning the nature and mechanism of atopic illnesses.

Although the skin is the shock organ in atopic eczemas and urticarias, the mucous membranes are the tissues involved in the most important clinical forms of atopic hypersensitiveness such as hay fever and asthma. Direct experimentation on these tissues, with a few exceptions, has been generally avoided. This was perhaps to be expected in view of their delicate nature, their inaccessibility, and their sensitiveness to physical and chemical stimuli, which ordinarily do not affect the skin.

The inadequacy of cutaneous hypersensitiveness as a criterion of mucous membrane sensitivity has, in the last few years, become more and more apparent. The need of more information concerning the nature and properties of mucous membranes as shock tissues in hypersensitiveness has become more urgent. It was this dearth of knowledge on a subject which seemed so fundamental in importance that prompted us to enter upon our investigations of mucous membrane hypersensitiveness.

The ophthalmic mucous membrane, because it was the most accessible of all, was chosen for the primary study. Moreover, it offered another

*From the Applied Immunology and Ophthalmology Clinics, Jewish Hospital of Brooklyn, New York.

†Read in abstract before the Society for the Study of Asthma and Allied Conditions, May 6, 1933, Washington, D. C.

advantage in that the extreme thinness of the conjunctivae permitted certain types of observations which were impossible on thicker mucous membranes and on the skin.

Because the Prausnitz-Küstner experiment had proved of such inestimable value in earlier studies of cutaneous hypersensitiveness, it seemed advisable to determine whether the same phenomenon could be employed to advantage in connection with mucous membranes. The specific questions to be answered were: First, could normal ophthalmic mucous membrane be passively and locally sensitized with a reagin-bearing serum; second, with what degree of regularity could such specific passive sensitization be accomplished in normal (nonatopic) individuals.

Two serums were employed in the present study. The first was obtained from a hay fever patient who was sensitive to ragweed pollen. He manifested a marked cutaneous reaction to an intracutaneous test with a ragweed extract in a dilution of 0.001 mg. N per c.c. The second came from a patient suffering with asthma and allergic rhinitis caused by rabbit dander. In this individual, a marked cutaneous reaction was obtained with an intracutaneous test of rabbit epithelium extract containing 0.001 mg. N per c.c.

Blood was drawn from each of these patients under sterile precautions. It was immediately defibrinated and centrifuged. The serums were drawn off, passed through Seitz filters, and kept without preservative in the ice box. They were used within a few days in order to avoid contamination and to prevent their becoming irritating as a result of aging.

As subjects for this study, 20 patients were selected from those attending the Out Patient Clinic in Ophthalmology because of some functional visual disturbance. Patients with an atopic personal or family history were not used.

The left conjunctiva of each subject was anesthetized by two instillations of a 2 per cent butyn solution into the conjunctival sac. One-tenth of a cubic centimeter of the sensitizing serum was then injected through a very fine needle under the ocular conjunctiva below the iris. At this site there resulted slight puffing of the conjunctiva, which was only temporary and was rapidly absorbed. The right conjunctiva was left untouched.

From two to ten days later 17 of the 20 patients returned for testing. In no instance did any evidence of the sensitizing injection remain.

In the 10 patients who had been injected with the ragweed serum, 1 drop of ragweed extract containing 0.1 mg. N per c.c. was instilled in both eyes. Within one to ten minutes a definite reaction developed in the left eye in every instance, while the right eye remained unaffected.

The reaction consisted of definite congestion of the ocular conjunctiva, most pronounced in its lower half. The lower palpebral conjunctiva was also usually involved, but to a less degree than the ocular.

TABLE I

RESULTS OF PASSIVE TRANSFER TO THE CONJUNCTIVAE OF 18 INDIVIDUALS

CASE NO.	SENSITIZING SERUM (0.05 C.C. INJECTED)	FIRST TEST			SECOND TEST		THIRD TEST		FOURTH TEST	
		INTERVAL AFTER SENSITIZATION IN DAYS	EXTRACT EMPLOYED (0.1 MG. N PER C.C.)	DEGREE OF REACTION	INTERVAL AFTER PRECEDING TEST IN DAYS	DEGREE OF REACTION	INTERVAL AFTER PRECEDING TEST IN DAYS	DEGREE OF REACTION	INTERVAL AFTER PRECEDING TEST IN DAYS	DEGREE OF REACTION
1	Ragweed	2	Ragweed	+	14	0				
2	Ragweed	6	Ragweed	++++	7	0	7	0		
3	Ragweed	2	Ragweed	++	7	0	5	0		
4	Ragweed	2	Ragweed	++++	7	0				
5	Ragweed	2	Ragweed	+	7	+				
6	Ragweed	6	Ragweed	++++	2	++++	7	++	3	0
7	Ragweed	9	Ragweed	+++	14	0				
8	Ragweed	2	Ragweed	++++	7	+	7	+	6	0
9	Ragweed	2	Ragweed	+	7	±				
10	Ragweed	2	Ragweed	++	7	±	2	0	4	0
11	Ragweed	7	Ragweed	++++	19	0				
12	Ragweed	2	Ragweed	++++	7	++	7	+	7	+*
13	Ragweed	2	Ragweed	+++						
14	Rabbit dander	2	Rabbit dander	++++						
15	Rabbit dander	6	Rabbit dander	++						
16	Rabbit dander	2	Rabbit dander	+++						
17	Rabbit dander	9	Rabbit dander	++++						
18	Rabbit dander	2	Rabbit dander	++						

*Positive reaction was obtained when test was repeated thirteen days later. After another interval of seven days a negative reaction was obtained on testing.

In strong reactions the blood vessels were considerably engorged and the sclera was reddened. The caruncle was usually inflamed. Itching and lacrimation accompanied the reaction, which at times became severe enough to necessitate the instillation of a few drops of epinephrin 1:1,000, which checked it at once. (A minute description of the reaction in all its details will be the subject of a later communication.)

To rule out any nonspecific factor as the cause of the positive ophthalmic reaction, an extract of timothy pollen of the same strength as the ragweed (0.1 mg. N per c.c.) was used as a preliminary test in both eyes in 3 patients. In no instance was there any positive response to this substance. A subsequent instillation of the ragweed extract resulted in definite reactions in the left eye in all patients, the right eye remaining unchanged.

In 5 cases in which the rabbit sensitizing serum had been injected into the left eye, the test was made with a rabbit epithelium extract containing 0.1 mg. N per c.c. All responded with a positive reaction in the left eye, the right remaining unaltered.

Complete desensitization of the passively sensitized conjunctiva was usually but not always effected as a result of the first test with the specific atopen. Subsequent repetition of the test in 12 ragweed sensitive patients after an interval of seven to nineteen days resulted in a positive reaction in 6 instances, but in all but one the response was much less than the original one. From two to seven days later, 4 of the latter patients when again tested were still found to be slightly sensitive. Sensitivity in one case was still present at the fifth test performed thirty-four days after the primary test (Case 12, Table I). The sixth test on the forty-first day was negative.

The fact that passive sensitization had been so easily effected in every instance, suggested the possibility of accomplishing the same end by repeated instillation of the sensitizing serum into the conjunctival sac instead of by injection. This was attempted in a small series of cases, but sensitization occurred in only a few patients and was present to only a very slight degree. The results with this technic were found to be unreliable for experimental purposes.

SUMMARY

1. Passive local sensitization of the normal conjunctivae in nonatopic individuals was readily effected in every one of 17 persons by subconjunctival injection of human serums containing reagins to ragweed pollen and rabbit dander.

2. The local sensitivity was shown to persist from one to five weeks or more and could be neutralized by one or repeated tests with the specific antigen.

PRELIMINARY REPORT UPON POLLEN CARBOHYDRATE AS A THERAPEUTIC AGENT FOR POLLEN ASTHMA*

A. H. W. Caulfeild, M.B., F.R.C.P.
Toronto, Canada

AS AN introduction I want to explain why the carbohydrate fraction of pollen was considered as a therapeutic agent rather than the usual protein-containing or protein-standardized pollen extracts.

It is not within the scope or purpose of this paper to review the published observations regarding the results which may be obtained by injecting specific substances such as the polysaccharides into sensitized animals. But certain of the observations by Avery and his coworkers stimulated some experimental studies by my colleagues and myself in animal sensitization using ragweed pollen extract† as the antigens.

Avery and Goebel[1] reported:

"1. When two chemically different carbohydrate derivatives are bound to the same protein, the newly formed antigens exhibit distinct immunologic specificity.

"2. When the same carbohydrate radicle is conjugated with two chemically different and serologically distinct proteins, both of the sugar proteins thus formed acquire a common serologic specificity.

"3. The unconjugated glucosides, although themselves not precipitable in immune serum, inhibit the reaction between the homologous sugar-protein and its specific antibody."

In a later study (III. Chemo-Immunological Studies on Conjugated Carbohydrate Proteins[2]) four interesting conclusions are made, one of which I shall quote. "3. The unconjugated glucosides, although themselves not capable of inducing shock, inhibit the anaphylactic reaction when injected immediately prior to the introduction of the toxigenic sugar-protein. The protective action of the glucosides disappears within two hours after injection. In order to elicit the phenomenon the carbohydrate must be the same as that combined in the sugar-protein complex." In other words, the inhibitive effect of the glucoside is specific.

These observations of Avery, Goebel and Tillett in 1929, that specific carbohydrate could under suitable circumstances inhibit both precipitation in immune serum and, *temporarily at least,* anaphylactic shock in sensitized animals suggested the idea that an investigation of the rôle

*Presidential address, read before the Tenth Annual Meeting of the Society for the Study of Asthma and Allied Conditions, Atlantic City, N. J., April 2, 1934.

†The carbohydrate fraction was prepared by E. T. Waters of the Department of Physiology of the University of Toronto; the method will be published when our experimental results are more complete. The material designated as pollen carbohydate material contained 1.6 per cent total nitrogen or less.

of pollen carbohydrate in whole pollen sensitized animals should prove of interest. Of interest, in that further light might be shed on the subject of anaphylaxis and possibly lead to some type of clinical application.

Our experiments with ragweed sensitized guinea pigs are not completed largely because of the difficulty we experienced in sensitizing guinea pigs with extracts of ragweed pollen. In other words, the percentage of animals (in any group, however sensitized) showing anaphylactic symptoms of severity (or dying) on reinjection was relatively small. So small that it was difficult to determine what the effect had been of an injection of pollen carbohydrate just previous to the reinjection of the original sensitizing whole pollen solution.

In no instance, however, did the injection of pollen carbohydrate into whole pollen sensitized animals induce any symptoms of shock.

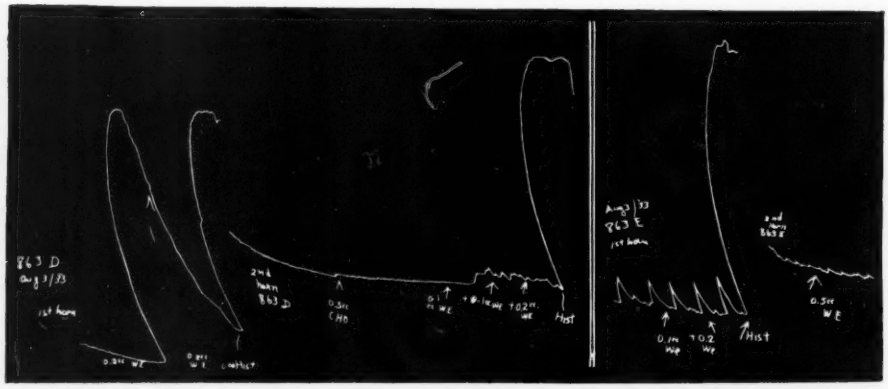

Fig. 1.—Uterine horn tracings in guinea pigs 863 D and 863 E. Guinea pigs 863 D and 863 E (females) of the same pure bred stock were sensitized with 1.0 c.c. W. E. (watery extract of ragweed pollen) subcutaneously on July 6, 1933.

Aug. 3, 1933, guinea pig 863 D, *1st horn* 0.2 c.c. W. E. contraction + (maximal). 0.2 c.c. W. E. added—no further effect. Allowed to relax, washed, 0.05 c.c. histamine gave contraction but no greater than initial contraction obtained with W. E. 0.2 c.c.

Second horn 0.5 c.c. Cho. for five minutes—no effect. 0.1 c.c. W. E. one and one-half minutes—no effect. 0.1 c.c. W. E. one and one-half minutes—no effect. 0.2 c.c. W. E. two minutes—no effect, washed. Histamine gave positive contraction.

Aug. 3, 1933, guinea pig, 863 E, female, duplicate of 863 D, illustrates the lack of constancy in animals injected at the same time with identical amounts.

First horn **negative to 0.2 c.c. W. E.: Histamine +.**

Second horn **negative to 0.5 c.c. W. E.: Histamine +.**

Because of the varying degrees of sensitization realized in any group of animals, it was found impossible to obtain anaphylactic death or severe shock in correlation to quantitative amounts in either the original or the secondary shocking doses. As a corollary no basis was available on which to estimate quantitatively the amount of carbohydrate necessary as a possible inhibitive.

An example is shown in Fig. 1 to illustrate the inhibitive effect of pollen carbohydrate on a sensitized guinea pig uterine horn to the

toxigenic effect of whole pollen solution. In this tracing the first uterine horn of guinea pig 863 D gives typical and maximal uterine contractions to 0.2 c.c. of whole pollen extract. This effect is inhibited in the second uterine horn by the addition of pollen carbohydrate to the bath before the addition of whole pollen extract. The second tracing shown of guinea pig 863 E shows complete lack of sensitization, although both received the same initial injection of pollen antigen. The results with these two guinea pigs serve as well to illustrate the difficulties encountered in inducing sensitization.

The evidence we obtained of the inhibitive effect in experimental anaphylaxis of ragweed carbohydrate when injected shortly before the reinjection of whole pollen solutions suggested that ragweed carbohydrate might have some clinical value in cases of asthma due to ragweed pollen.

All patients with asthma due wholly or in part to ragweed pollen encountered during the autumn of 1933 were given injections of pollen carbohydrate, and an attempt was made to estimate its therapeutic effect. This is admittedly difficult for many reasons, among which the following seem to be important.

In the summary of cases cited, the date of onset varied to such a degree that one is left without a satisfactory explanation as to why this varies so in different individuals living in the same geographical area. The severity of the attack when first seen can be fairly accurately estimated on clinical grounds, and the amount of adrenalin necessary to control it recorded, but there are no means by which one can foretell how long the attacks are likely to continue or, in other words, no standard by which different methods of treatment can be contrasted one against the other. One can only gain an impression once a new preparation is used by comparing how much adrenalin was necessary before, during, and after, to control or prevent the attacks. In presenting the summaries of these cases an attempt has been made to bring out these points.

For clinical work the carbohydrate material was dissolved in saline solution 2.5 mg. per cent (250 mg. Cho. dissolved in 100 c.c. saline) and diluted as detailed in the case histories.

SUMMARY OF CASES

CASE 1.—G. O. A. (Skin test with Cho.* strongly positive.) Had finished a course of desensitization against grass and autumnal pollens on Sept. 18, 1933, with fairly satisfactory success. On Sept. 28, 1933, he reported a severe asthmatic attack beginning the day before, which was partially relieved by self-administered adrenalin min. 5 at 8 P.M. No more adrenalin was taken because of marked muscular and general discomfort.

	2:20 P.M.	Cho.* (1:100) 0.25 c.c. plus adrenalin 0.1 c.c.—definite but not complete relief.
	2:50 P.M.	Cho. (1:100) 0.35 c.c. plus adrenalin 0.05 c.c.—further improvement in four minutes.
	3:10 P.M.	Cho. (1:100) 0.5 c.c. No adrenalin—marked relief. No pallor, as usual after adrenalin, climbed two flights of stairs after third dose.
Sept. 29,	10:30 A.M.	Reported he had remained free until about 7 A.M. Since then attacks increased in severity. Now in marked distress.
	10:30 A.M.	Cho. (1:10) 0.1 c.c. plus adrenalin 0.1 c.c.—relief marked.
	10:58 A.M.	Cho. (1:10) 0.15 c.c. plus adrenalin 0.05 c.c.
	11:30 A.M.	Cho. (1:10) 0.2 c.c. No adrenalin—left in complete relief.
Sept. 30,	11:30 A.M.	Reported he had felt slight distress early in the morning which seemed to pass off. Cho. (1:10) 0.25 c.c., no adrenalin.
Oct. 2,	11:45 A.M.	Reported he had remained free until about 4 P.M on Oct. 1. Slow recovery with increase again beginning about 7 A.M. today. Now in considerable distress. Cho. (1:10) 0.3 c.c., no adrenalin—improvement in fifteen minutes.
	12 NOON	Cho. (1:10) 0.3 c.c., plus adrenalin 0.05 c.c.—seemed entirely free.
Oct. 10.	11:00 A.M.	Reported free until night of Oct. 8. Now in moderate asthmatic distress. Cho. (1:10) 0.3 c.c. No adrenalin—marked improvement in fifteen minutes—asthma barely noticeable on walking.
	11:15 A.M.	Cho. (1:10) 0.3 c.c. plus adrenalin 0.05 c.c.—immediately asked how much adrenalin he had had on account of general discomfort.
	11:30 A.M.	Cho. (1:10) 0.3 c.c., no adrenalin—left but was not perfectly free.
	3:40 P.M.	Reported attack began twenty minutes before. Now in severe attack. Cho. (1:10) 0.3 c.c. plus adrenalin 0.1 c.c.—marked improvement.
	4:15 P.M.	Cho. (1:10) 0.3 c.c. No adrenalin. Felt "better than any time today."

Cho.* = ragweed carbohydrate solution.

Slight attacks occurred on different days, the eleventh, fourteenth, and seventeenth of October. Two or three injections of Cho. were given on each occasion without the addition of adrenalin. Reported later in November that he had remained entirely free.

Comment: The addition of very small amounts of adrenalin seemed as with most cases to be necessary to obtain complete freedom if the attack was very severe. In slight attacks there was a very evident improvement by injection of Cho. alone.

January, 1934, an attack of asthma was not influenced by ingestion of ragweed Cho. but controlled by suitable dog hair preparation to which patient was also sensitive.

CASE 2.—Mrs. D. Referred by and treated in conjunction with Dr. G. C. Anglin. Patient had been treated routinely against autumn pollens with marked relief of her hay fever. (She was also sensitive to certain of the epidermals.) Admitted to hospital Oct. 1, 1933, with a history of severe asthmatic attacks which had begun a week previously—so severe she could not report to her hospital clinic. Administration of repeated doses of adrenalin in 5 mm. and 7 mm. doses was only partially effective on account of heart palpitation, etc. On Oct. 2 she had had continuous and severe attacks throughout the day. At 8 P.M. in very marked distress, Cho. (1:100) 0.3 c.c., plus adrenalin 0.15 c.c., gave some relief in ten minutes. At 8:20 P.M. 0.5 c.c. Cho. plus 0.1 c.c. adrenalin produced such marked relief in four minutes that she could talk with freedom. At 8:45 P.M. 0.75 c.c. Cho. with no adrenalin left her more comfortable than at any time. She slept perfectly but began to experience slight respiratory distress by following afternoon.

Oct. 3, 3:35 P.M. Cho. (1:10) 0.07 c.c. plus 0.15 c.c. adrenalin.
4:00 P.M. Cho. (1:10) 0.15 c.c. plus 0.1 c.c. adrenalin resulted in complete relief.

Oct. 4, 3:25 P.M. In moderate attack of asthma. Reported she had remained perfectly free until about 7 A.M., when distress began, and was relieved but slightly by ephedrine.
Cho. (1:10) 0.15 c.c., plus 0.15 c.c. adrenalin, gave considerable relief but cardiac action uncomfortable to patient and perceptible on observation.
3:55 P.M. Cho. (1:10) 0.15 c.c., no adrenalin, marked improvement.
4:20 P.M. Cho. (1:10) 0.15 c.c., no adrenalin, slight wheezing, only perceptible by auscultation.

Oct. 5, 11:15 A.M. Reported no real asthma but slight wheezing during early morning; now had slight sense of constriction.
Cho. (1:10) 0.2 c.c., no adrenalin.

11:45 A.M. Cho. (1:10) 0.2 c.c., no adrenalin.
12:00 NOON Cho. (1:10) 0.2 c.c., no adrenalin, had sense of complete freedom.

From this date on, no adrenalin was administered with the Cho. Last set of two injections given on Oct. 10; since then she has remained free.

CASE 3.—F. F. W. (Skin tests Cho. positive.) Seen in consultation with Dr. L. O. L. Skeeles, Oct. 25, 1933. Patient had been confined to bed or house for about past three weeks with continuous attacks of asthma which had been steadily progressing, especially at night. History suggested hay fever asthma, in that he had had almost yearly attacks in autumn or late summer for the last nine years with relatively rare attacks at other seasons. His physician had been using adrenalin in doses of from 0.5 c.c. to 0.75 c.c. and felt that he was making no headway against the condition. On Oct. 28 skin tests showed positive reaction to ragweed fraction (albumin proteose) and ragweed Cho. (6 mm. by 8 mm. and 5 mm. by 6 mm. wheals plus areolas respectively). Slight reaction to other substances.

Cho. 1:100 dilution 0.05 c.c. plus 0.05 c.c. adrenalin—relief in fifteen minutes.

Oct. 29. Cho. 1:100 dilution 0.1 c.c. plus 0.05 c.c. adrenalin—moderate relief in three minutes.
Cho. 1:100 dilution 0.15 c.c. (twenty minutes later)—continued free until 4:30 A.M.

From this time on, increasing daily or twice daily doses of Cho. without addition of adrenalin were given by Dr. Skeeles with no further night attacks and with continued improvement. Fully free of physical signs by Nov. 10. Relief following injections was frequently very definite.

Comment: It is difficult to evaluate influence of advancing season and injections except in early doses and their immediate effect.

CASE 4.—G. I. Asthma since 1928—always severe from late summer on. Skin tests never strongly positive, but frequently ragweed pollen or fractions were positive. Yearly treatments with ragweed extracts seemed to patient to be beneficial, but he always had asthma throughout September, and in 1932 this continued into December. The last tests (1933) with different ragweed extracts and Cho. by intradermal method were negative. Began to have asthma about the middle of September and had to self-administer adrenalin in 0.35 c.c. to 0.7 c.c. doses at frequent intervals.

The following is illustrative:

On Sept. 18 he had his third closely spaced injection of Cho. with 0.05 c.c. of adrenalin added to the first dose at 3 P.M., and on Sept. 19

at 4:45 P.M. was again in distress but stated he had remained free until 7 P.M. of the eighteenth, since when he had taken six injections of adrenalin in 0.5 c.c. doses.

Sept. 19, 4:45 P.M. Cho. dilution 1:10 0.2 c.c., plus 0.15 c.c. adrenalin, gave some relief.

5:00 P.M. Cho. dilution 1:10 0.25 c.c., plus 0.1 c.c. adrenalin, gave full relief, which persisted until about 7:30 A.M. of the twentieth.

Following this the maximum dose of adrenalin combined with the Cho. was 0.1 c.c., and on different days he remained sufficiently free to cease his self-administration of adrenalin. The last note is that on Sept. 27, he had taken no adrenalin since Sept. 25.

Comment: The effect of the Cho. was striking in that such small doses of adrenalin were effective and for this case relatively lasting—particularly in contrast to the difficulties experienced other years. His asthma, however, was not regarded as due solely to pollens. A sudden change to cold weather would almost invariably bring on severe attacks.

CASE 5.—M. H. (Skin tests with Cho. strongly positive.) Yearly treatments since 1929. Asthma before treatments was chief cause of consultation. Nearly every year, despite treatments, he would have periodic attacks of asthma. In 1932 coseasonal treatments continued into October with additional use of adrenalin and distress of a type noticeable even in December. In 1933 Cho. was used in addition from Aug. 30 until Sept. 21 when treatments were stopped because he was entirely free. Dosage was combined with the ordinary solutions and Cho. plus adrenalin in 0.05 c.c. amounts on four occasions. Any asthma present was very slight.

Comment: To what extent the Cho. played a part in making 1933 the best year he had ever had can, of course, not be determined and can only be added to the favorable impression afforded by the other cases. Certain of the injections of Cho. gave definite local reactions of mild degree.

CASE 6.—C. F. A. (Skin tests to Cho. positive.) Yearly treatments since 1922. Despite treatment, patient always experienced periodic distress during a season of moderate severity. He seldom had definite asthma but had a sense of persisting chest constriction. Extremely sensitive to routine pollen solutions so that by Aug. 21, 1933, after fifteen injections he had reached the second bottle of tenfold dilution (complete set being four bottles of tenfold dilutions). Returned from holidays on Oct. 13 and experienced a return of nasal irritation and chest discomfort. Cho. dilution 1:100 0.15 c.c., caused slight local reaction and itching. Under self-administration of 0.15 c.c. of 1:200 dilution he reported that local irritation followed the first injection but none fol-

lowed the second or third with half-hour intervals, and he experienced relief from the sense of chest constriction. Adrenalin could not be used in this case on account of cardiac disease. Reported he was entirely free by Oct. 31.

Comment: The effect of Cho. was not decisive but was preferred by this patient to the usual ragweed solutions used other years and certainly could be self-administered more safely.

CASE 7.—M. E. J. Since presentation of this article, the following results were obtained during the early part of the 1934 season and seem to be worth insertion. Patient was skin positive to ragweed pollen and other pollens and proteins. Since 1927 asthmatic attacks despite treatment had been severe and required hospitalization for four of the years, the attacks terminating on the following dates: November 5, October 25, November 14, November 8, September 25, and October 24, respectively. This year the patient reported on September 7 that she was self-administering adrenalin on an average three times daily in 9 minim amounts. Following an office treatment, she was supplied with ragweed Cho. for self-administration, and reported as follows:

1:30 P.M.	0.2 c.c.	Cho. 1:100.	No asthma.
3:30 P.M.	0.2 c.c.	Cho. 1:100.	No asthma.
5:30 P.M.	0.2 c.c.	Cho. 1:100.	No asthma.
7:30 P.M.	0.2 c.c.	Cho. 1:100.	No asthma.
9:30 P.M.	0.2 c.c.	Cho. 1:100.	Slight asthma at 9:40 P.M. for a few moments.
2:30 A.M.	0.2 c.c.	Cho. 1:100.	When she awoke with slight distress.

On September 8, she reported, she had to take adrenalin at 12:30 P.M. Later ragweed Cho. was supplied for self-administration with approximately similar reports.

CONCLUSION

The date of onset of pollen hay fever asthma and its duration and severity vary so much in individual cases that it is difficult to contrast accurately the different methods of treatment. By the use of pollen carbohydrate solution the impression was left that the asthmatic attacks were relieved more satisfactorily and quickly, and with much smaller amounts of adrenalin than by any other specific method of treatment.

I wish to acknowledge the cooperation given us by Knapp and Knapp in helping to provide the large amounts of ragweed pollen necessary to make the different ragweed preparations.

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FURTHER OBSERVATIONS CONCERNING THE EFFECT OF POLLEN THERAPY UPON CUTANEOUS SENSITIVENESS IN LATE HAY FEVER*

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IT HAS been our impression, as previously stated[1] and shared by many others, that cutaneous reactions in the average hay fever patient, obtained by intracutaneous test at the conclusion of a season of intensive treatment, differ very little from those obtained at the beginning of the season; that changes in sensitiveness to a greater degree can be traced to the usual seasonal loss of potency of the extract; that longer periods of treatment would tend to influence the skin toward a lessening of sensitiveness; and that this diminished response would be very gradual and often imperceptible.

This impression is not shared by others[2, 3, 4] who maintain that the sensitiveness of the skin as measured by the reaction to cutaneous pollen tests definitely diminished, frequently to the point of absolute disappearance, even in a single season of treatment; indeed, a method of measuring the increase of pollen therapy dosage by the rapidity and extent of this diminution of cutaneous sensitiveness has been suggested, the same extracts being employed for tests at the beginning and the end of the season.

In order to study the changes of cutaneous sensitiveness in hay fever over a longer period of time, a group of 30 patients with ragweed hay fever were chosen. They had received the perennial type of specific pollen therapy during the previous year, and the dosage had been high and fairly constant throughout the year with no constitutional reactions recorded. Before each monthly injection of the current ragweed extract, the patients were tested intracutaneously with dilutions of this extract containing 0.0001 mg., 0.001 mg., and 0.01 mg. N per c.c. concentration. At the height of the reaction, permanent records were made by outlining the wheal and erythema with ink and tracing directly upon transparent paper. Charts 1 and 2 show these tracings. This procedure was continued from February, 1933, to October, 1934. Three separate and distinct extracts were used, beginning with the 1932 ragweed extract, changing to the 1933 extract in April, 1933, and to the 1934 extract in April, 1934. At the conclusion, 23 complete sets of tracings had been obtained, and the records of two entire hay fever seasons are thus included in this report.

*From the Division of Immunology, Department of Medicine, New York Post Graduate Medical School and Hospital.

By referring to the charts it will be noted that there is very little change in the cutaneous response to intracutaneous tests with ragweed extract dilutions from February, 1933, to September, 1934, unless possibly to the tests with the weakest concentration (0.0001 mg. N per c.c.), which varies slightly from month to month. However, when new and

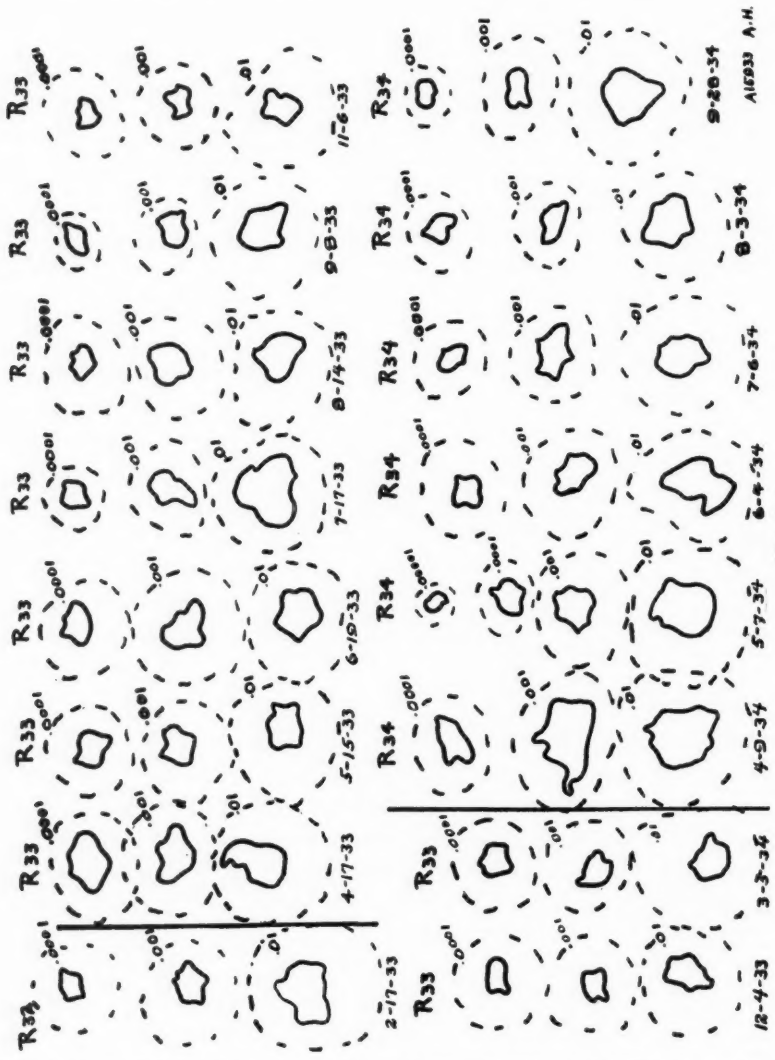

Chart 1.

Charts 1 and 2.—Tracings of reactions of intracutaneous tests with ragweed extract dilutions at monthly intervals.

R = Ragweed.
32 = 1932.
33 = 1933.
34 = 1934.

.0001 = 0.0001 mg. N per c.c.
.001 = 0.001 mg. N per c.c.
.01 = 0.01 mg. N. per c.c.

freshly prepared extracts were introduced at two testing periods (April, 1933, and April, 1934), there appeared a distinct increase in the wheal and erythema reactions to all concentrations of the extract, but this

gradually subsided as the current extract aged. In no case during this period had reactions disappeared or even greatly diminished, in spite of the fact that the clinical results in this group were excellent and a high level of dosage was maintained. Charts 1 and 2 are typical of the entire group.

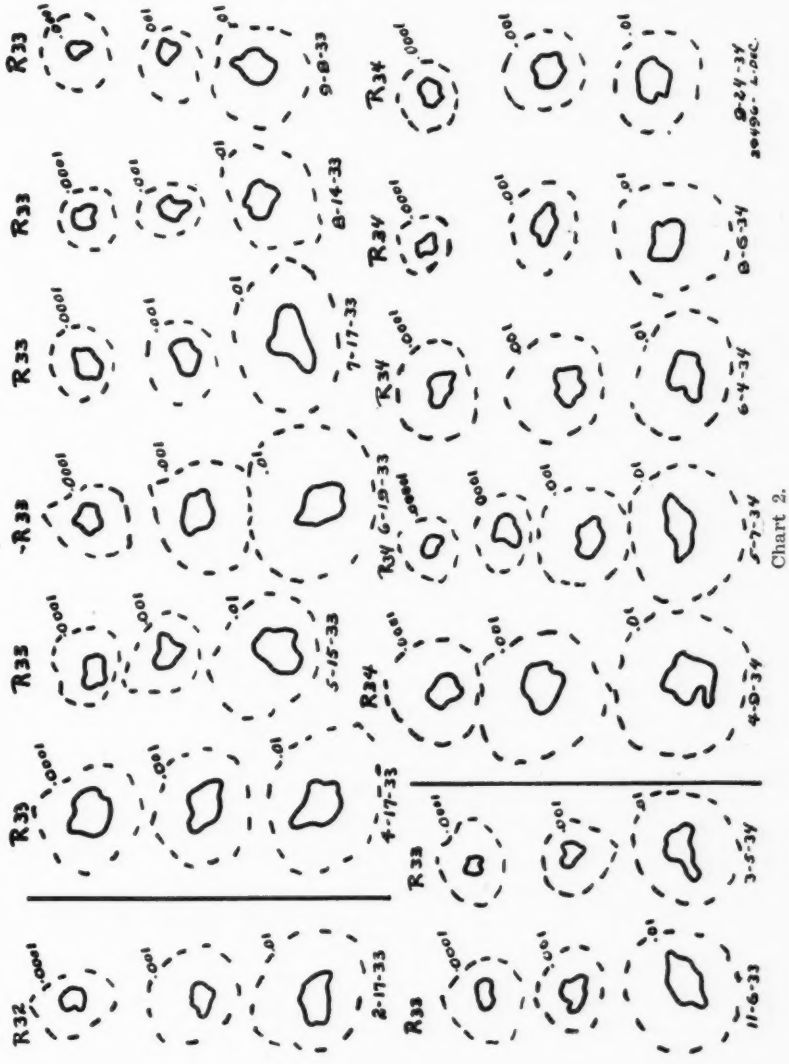

Chart 2.

DISCUSSION

It is evident that within a period of two years prolonged treatment of late hay fever with high doses of ragweed extract is not accompanied by any appreciable diminution of skin sensitiveness when determined by the intracutaneous method of testing. In a group of 23 late hay fever patients, tests at the beginning and end of a twenty-month period com-

pared favorably in the degree of cutaneous response, very slight variations in the interval being noted. The substitution, in tests, of freshly prepared extracts at widely separated intervals caused a distinct increase in the activity of the cutaneous response, noticeably above the level maintained by the same extracts tested when not so recently prepared. Any marked diminution in sensitiveness as the result of treatment seems to be the exception rather than the rule. In other words, in evaluating differing results by test, it should be emphasized that it is the skin that is the constant factor; the extract that is the variable factor, and not the reverse. In these tracings a gradual but perceptible deterioration of the extracts is discernible. Accordingly, in the perennial type of treatment especially, it is advantageous to use extracts prepared at frequent intervals so that any loss of potency may be replaced before it has progressed to any appreciable extent.

CONCLUSIONS

Cutaneous sensitiveness in hay fever varies little as a result of one or two years of perennial treatment.

The variations in the degree of activity by test, often noted during a course of treatment, are, in the main, due to changes in the potency of the extract.

This deterioration in an extract must be taken into account when the classification of a hay fever patient as to the degree of sensitiveness is attempted.

In the perennial type of treatment, preparation of fresh extracts at frequent intervals during the year will tend to eliminate this variable factor.

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THE PROTEIN CONTENT OF EXTRACTS OF VARIOUS ALLERGENS*

Methods for Determining Small Amounts of Protein

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CONSIDERABLE interest has recently been taken in the problem of the nature of allergenic compounds and in the chemical standardization of solutions used in the allergy clinic. Much of this work has dealt with nitrogenous compounds, particularly with proteins. We believe that further knowledge of the amounts of protein and of protein-like compounds in solutions used in the study and treatment of allergic conditions not only will be of value in itself, but also may help in solving some of the problems met with in the clinic. We have therefore carried out a series of determinations of some of the nitrogen fractions in solutions of various allergens. The results of these determinations and a discussion of some of their possible applications are given below.

Cooke and his various coworkers[1, 2, 3] have advanced evidence tending to show that the active material in some of the pollens is a protein or at least is protein-like in its nature. Their conclusions have not been accepted by all workers,[4] but they must influence the direction taken by further chemical investigations. In the present study we have determined the protein content of weakly alkaline solutions prepared not only from pollens, but also from other allergens. Although little work has been done on the chemical nature of the active material in many of these substances, we believe that the results of chemical determination of the amounts of protein and other nitrogenous compounds extracted from them by a standardized technic are of sufficient interest to justify the present report.

For the purposes of this work we used extracts prepared as follows. The crude material was placed in a Soxhlet apparatus and the fat extracted with petroleum ether. The dry, fat-free material was then treated with Coca's extracting fluid[5] (sodium chloride 25 gm., sodium bicarbonate 13.75 gm., 21.05 c.c. of 95 per cent phenol and water to make 5 liters) and placed in the ice box overnight. To some of the substances, toluol was added before extracting with Coca's solution to inhibit the growth of bacteria and molds. The mixture was strained

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through a Buchner funnel to remove particulate matter, and sterilized by Seitz filtration. The chemical analyses were carried out on the sterile filtrate.*

In every instance the material had been prepared for testing and treating patients. It was therefore desirable to take only small amounts for the analyses so that as much as possible might be available for use in the clinic. This fact led us to experiment with various micromethods, and eventually to develop precedures which differ somewhat from those which have been previously described. We feel that these methods may be of use to others, and shall therefore give a detailed description of them.

Recently Stull, Cooke and Tennant[6, 7] have proposed that the protein content of pollen extracts be determined by a regular Kjeldahl method on the precipitate obtained by treatment with phosphotungstic and hydrochloric acids.[6, 7] The first method we developed was based closely on their procedure. The departures made from their technic consisted of reduction of the amount of solution taken for an analysis and of substitution of a micro- for a macro-Kjeldahl determination.

There is a legitimate criticism which can be directed against the phosphotungstic acid method. This acid is a good precipitant for proteins, but is not specific for such compounds. (On this point see discussion by Dr. Robert A. Cooke of the paper of Spain and Newell.[15]) Other substances are removed from solution when it is used, and many of these contain nitrogen. Among them are proteoses, peptones, most of the naturally occurring aminoacids, and many nitrogen bases; creatinine is included in the latter group. In studies of the sort planned it seemed desirable to use a method more specific for protein if one were available.

Trichloracetic acid seemed to be well adapted for the purpose, for it has been frequently recommended for determining protein in biological material.[8, 9, 10] Its effect upon alkaline solutions prepared as described was therefore investigated. Complete recovery of precipitated material was somewhat difficult when only very small amounts of protein were present, but it was found that if the precipitation was carried out in the presence of methyl alcohol (about 60 per cent by volume) this difficulty could be avoided. A number of preliminary experiments were done to test the accuracy of this procedure. To begin with, diluted, blood serums of known protein content were analyzed with very satisfactory results. After removal of protein from the allergen extracts by means of trichloracetic acid, the filtrates were tested with sulphosalicylic acid—a very sensitive reagent for showing the presence of protein in solution[11]—and negative reactions regularly obtained. A few determinations were carried out in which the effect of methyl alcohol upon the precipitation of large amounts of protein was studied. With most of the

*The solutions of egg white and glue were made up directly in Coca's solution and Seitz filtered. The milk solution consisted of the whey derived from carefully skimmed milk which had been treated with rennin and the curd removed. It was Seitz filtered before analyzing.

extracts, identical values were obtained when trichloracetic acid was used by itself and when methyl alcohol was also present. In an extract prepared from LePage's glue, however, part of the precipitate formed by trichloracetic acid was redissolved by methyl alcohol. This effect suggests strongly that methyl alcohol may prevent the precipitation of proteose and peptone, since glue is very rich in compounds of this type, and thus its use in conjunction with trichloracetic acid renders the treatment more specific for native proteins than is the acid by itself.

EXPERIMENTS

Apparatus.—(1) Conical centrifuge tubes of pyrex glass which will contain 50 c.c., graduated at 10 c.c. (2) Micro burner. (3) Ring stand with adjustable clamp to hold centrifuge tubes. (4) Small watch glass.

Reagents.—(1) Phosphotungstic acid solution: 10 gm. pure phosphotungstic acid dissolved in 100 c.c. of a mixture containing 10 c.c. of concentrated hydrochloric acid and 90 c.c. of distilled water. (2) Concentrated hydrochloric acid. (3) Twenty per cent trichloracetic acid. (4) Absolute methyl alcohol. (5) The dilute (1:1) sulphuric acid and phosphoric acid digestion mixture of Folin and Wu.[12] (6) Ten per cent Rochelle salt solution. (7) Nessler's solution prepared according to the directions of Folin and Wu.[12] (8) A standard solution of pure ammonium sulphate containing 0.1414 gm. in a liter. One c.c. of this solution contains 0.03 mg. nitrogen.

METHOD.—*Phosphotungstic acid precipitation:* Place 2 c.c. of the extract in a 50 c.c. centrifuge tube. Add 1.5 c.c. concentrated hydrochloric acid and 0.5 c.c. of the phosphotungstic acid solution. Mix and centrifuge. Decant completely, drain, and discard the supernatant fluid. Wash the precipitate with 4 c.c. of a mixture containing 3 c.c. of concentrated hydrochloric acid and 1 c.c. of the phosphotungstic acid solution if desired. Our experiments indicated that this precaution was not necessary.

Trichloracetic acid precipitation: Measure 2 c.c. of the extract into a 50 c.c. centrifuge tube. Add 2 c.c. of 20 per cent trichloracetic acid. Heat in a boiling water-bath thirty to sixty seconds. (This step can be omitted when fairly large amounts of protein are present.) Let cool. Add 6 c.c. of absolute methyl alcohol and mix. Centrifuge the tube at a high speed for thirty minutes. Decant completely and drain. Washing is unnecessary. If less than 2 c.c. of the extract is available for either method, less may be used, and enough water added to give a total volume of 2 c.c.

Oxidation: The method is exactly the same when phosphotungstic acid or trichloracetic acid is used. To the centrifuged precipitate in the tube add 2 c.c. of the dilute oxidizing mixture of Folin and Wu. Heat the tube carefully over a low flame until the precipitate has dissolved. The

tube must be shaken constantly to avoid bumping. Solution of the trichloracetic acid precipitate takes place almost instantly, but the precipitate given by the phosphotungstic acid dissolves much more slowly. After solution has occurred, clamp the tube in a slanting position and heat with a micro burner until the water has boiled away and white fumes appear. Place the tube in a vertical position, cover with a watch glass, and continue heating until the brown or yellow color has entirely disappeared. Let cool until the fumes have almost wholly subsided. Tilt the tube and dilute with about 5 c.c. of distilled water from a medicine dropper, pouring the water down the side of the tube. The water should be added a drop at a time at first to avoid spattering, and more rapidly as soon as the violent reaction has slackened. Let the contents of the tube cool. If the amount of oxidizable material is very large, a crystalline precipitate may form at this point; this dissolves if it is stirred with a small glass rod, which should be rinsed into the tube. Bring the volume up to 10 c.c. with distilled water. Centrifuge if a turbidity is present. For Nesslerization, pipette 5 c.c. into a second tube graduated at 10 c.c., add 1 c.c. of 10 per cent Rochelle salts and dilute to 10 c.c.

Nesslerization: Prepare in tubes graduated at 10 c.c. a series of standards containing 0.03 mg., 0.06 mg., 0.09 mg., and 0.15 mg. of nitrogen. These amounts are contained in 1 c.c., 2 c.c., 3 c.c., and 5 c.c. of the standard ammonium sulphate solution described above. Add to each tube of standard thus set up 1 c.c. of 10 per cent Rochelle salts and 1 c.c. of the dilute oxidizing reagent of Folin and Wu. Make up each to 10 c.c. Add 15 c.c. of Nessler's reagent prepared according to the directions of Folin and Wu simultaneously to the standards and to the tube containing the aliquot of the oxidized precipitate. Compare the color of the latter with that of the standard nearest to it in tint in a colorimeter. Calculate the protein concentration from the following formulas:

$$\text{Protein nitrogen in 1 c.c. of extract} = \frac{\text{reading of standard}}{\text{reading of unknown}} \times \text{value of standard.}$$

Percentage of protein = protein nitrogen per c.c. $\times$ 100 $\times$ 6.25.

If less than 2 c.c. of the original solution was taken, a factor must be introduced in the calculation. If the color of the unknown varies too widely from the above standards as set up, comparison is impracticable. It is better to repeat the analysis using smaller or larger amounts of the allergen solution, although standards of greater or less strength than those described can sometimes be used successfully.

DISCUSSION

In carrying out the two procedures described, it was found that the precipitate formed by trichloracetic acid was very easily oxidized, but

that the one obtained with phosphotungstic acid was sometimes decomposed only with great difficulty. The explanation seems obvious: many nonprotein compounds are precipitated by phosphotungstic acid, and some of these are very rich in carbon. It seemed desirable therefore to run a control upon the phosphotungstic acid method. These control de-

TABLE I

MATERIAL	NOTES	TOTAL NITROGEN*	PROTEIN NITROGEN (BY DIFFERENCE)†	NITROGEN IN PHOSPHOTUNGSTIC ACID PRECIPITATE	NITROGEN IN TRICHLORACETIC ACID PRECIPITATE
Ragweed pollen, R 32		0.404	0.105	0.104	0.018
Ragweed pollen, R 333		0.337	0.180	0.160	0.047
Ragweed pollen, R 335		0.395	0.209	0.176	0.038
Timothy pollen, T 33 A		0.270	0.140	0.152	0.105
Timothy pollen, T 33 B		0.299	0.147	0.148	0.101
Timothy pollen, T 34 A		0.441	0.286	0.290	0.200
Timothy pollen, T 34 B		0.429	0.290	0.263	0.160
Timothy pollen, T 34 spec.	1 filtration	0.339	0.269	0.224	0.150
Timothy pollen, T 34 spec.	4 filtrations	0.323	0.244	0.178	0.098
Timothy pollen, T 34 spec.	9 filtrations	0.267	0.109	0.100	0.035
Cockleburr pollen	Roosevelt Hosp. 1933‡	0.118	0.055	0.048	0.004
Corn pollen	Roosevelt Hosp. 1933‡	0.120	0.055	0.054	0.020
Pigweed pollen	Roosevelt Hosp. 1933‡	0.149	0.049	0.047	0.009
Caddis fly, SFH 32	Hydropsyche, 1 filtration	1.65	0.150	0.150	0.076
Caddis fly, SFH 32	2 filtrations	1.385	0.144	0.144	0.046
Caddis fly, SFH 34	Hydropsyche	1.417	0.433	0.429	0.103
Caddis fly, SFH 342	Hydropsyche	2.206	0.606	0.585	0.260
Caddis fly, SFM 32	Macronema	1.16	0.100	0.097	0.025
Caddis fly, SFM 34	Macronema	1.333	0.365	0.400	0.122
May fly, 1934		1.128	0.383	0.405	0.036
Indian gum, 1934	Karaya	0.022	0.011	0.006	0.004
Cocoanut, 1933		1.304	1.258	1.244	0.945
Walnut, 1933		0.207	0.046	0.043	0.023
Almond, 1933		5.17	4.77	4.80	4.62
Pecan, 1934		0.074	0.007	0.014	0.009
Milk, 1934		1.364	0.998	1.03	0.708
Egg white, 1934		1.67	1.614	1.620	1.35
LePage's glue		6.45	6.19	5.45	2.73
House dust		0.282	0.028	0.036	0.007

*Results are given in terms of milligrams of nitrogen per cubic centimeter.

†Total nitrogen less nonprotein nitrogen.

‡These were solutions supplied by the Department of Allergy, Roosevelt Hospital, New York, N. Y., for use in our Allergy Clinic.

terminations were done by a technic resembling an early procedure of Cooke and Stull.[6] The total nitrogen content of each original extract was determined by a standard method.[13, 14] The residual (nonprotein) nitrogen in the supernatant fluid after precipitation with phosphotungstic acid was also determined by a similar technic. The difference

between these values furnished a measure of the "protein nitrogen" present—specifically, nitrogen precipitated by phosphotungstic acid.

There are objections to the "protein nitrogen" value obtained by this control method. One is inherent in the method of calculating the protein nitrogen. It depends upon a calculated difference between two determined values. When the difference is small in proportion to the two quantities actually determined—in the present discussion, when much nitrogen but little protein is present in the solution—the error in such a figure is very high. Two per cent is fairly close agreement between duplicate colorimetric determinations. Two separate colorimetric readings serve as the basis for calculating the control protein nitrogen values in these experiments. There were a number of studies in this series in which errors of only 2 per cent in each determination might have resulted in an error of 50 per cent in the difference between them, and hence in a 50 per cent error in the protein nitrogen when calculated by difference.

Another objection to the control method was practical rather than theoretical. A few of the solutions studied were so rich in carbohydrate and so poor in nitrogen that they could not be oxidized by the micro-method unless very small amounts of extract were taken for study. Such a procedure greatly increased the error in each nitrogen determination, and therefore in the figure based upon the difference between them. The foregoing discussion makes it clear that for both theoretical and practical reasons the control method was less satisfactory than was the direct analysis of the phosphotungstic acid precipitate. It did serve, however, as a rough check upon the results obtained by the latter.

The figures obtained on the various extracts are given in Table I. A study of these figures shows several facts. The direct micro-oxidation of the phosphotungstic acid precipitate appears to have been quite satisfactory, for this nitrogen value agreed closely with the "protein nitrogen" as calculated from the difference between the figures for total and nonprotein nitrogen. The exceptions—notably some of the pollens—were found in extracts very rich in carbohydrate, and hence, as discussed in an earlier paragraph, not well suited to protein nitrogen determinations by the control method.

The nitrogen in the precipitate formed by trichloracetic acid and methyl alcohol was always less than that in the material precipitated by phosphotungstic acid and hydrochloric acid, as would be expected from the more highly specific nature of the former precipitants. The ratio between the two sets of figures—nitrogen precipitable by phosphotungstic acid and nitrogen precipitable by trichloracetic acid—varied greatly in different preparations as would be expected. Almost all of the nitrogen precipitated by phosphotungstic acid from the almond extract was apparently in the form of true protein, for the trichloracetic acid precipitate gave practically the same nitrogen value. A fairly close agree-

ment is also found in the egg white solution. On the other hand only a relatively small proportion of the phosphotungstic acid precipitate from the ragweed extracts was of purely protein nature. The balance must have been made up of one or more of the nonprotein nitrogen compounds (proteoses, peptones, aminoacids, nitrogen bases, etc.), which are precipitated by phosphotungstic acid but are not removed from solution by trichloracetic acid.

The results of repeated Seitz filtration of the timothy extract are interesting. (The results of such treatment have been reported by Stull and others,[7] and by Spain and Newell.[15]) The amounts of nitrogen precipitated by either phosphotungstic acid or trichloracetic acid decreased as the number of filtrations increased. Eight filtrations reduced the nitrogen precipitable by phosphotungstic acid 0.124 mg. per c.c.; simultaneously the nitrogen precipitable by trichloracetic acid was reduced 0.115 mg. per c.c. These results are identical within the limits of error of such calculated differences. Repeated Seitz filtration therefore appears to remove protein from solution, for that is the substance precipitated by both acids.

Perhaps a word should be said about the application of these studies to the standardization of extracts for clinical use. Such a discussion must be approached with great caution for a number of reasons. Strictly speaking, the chemical standardization of an active principle can properly be made only after the chemical nature of that principle has been fully established. In studies of some of the pollens, considerable evidence has been advanced tending to prove that the active compound is a protein, and in one paper recently published[3] it was apparently shown that the active substance of one of them, timothy, could be precipitated with trichloracetic acid. On the basis of this evidence one would probably be justified in using an analysis of the trichloracetic acid precipitate in standardizing timothy pollen.

We do not believe, however, that this fact warrants a general extension of the method to other substances used clinically, which have not as yet been carefully studied by chemical methods. The active principle may be protein-like in nature, but not belong to the group of true proteins. In glue, for example, one of the proteoses or peptones may be the compound to which people are commonly sensitive. If that were the case, a standardization based upon an analysis of the trichloracetic acid precipitate would be misleading.

Another possible fallacy in standardizations based on results of the type given in this paper lies in the specific nature of allergic sensitivity and the nonspecific nature of chemical analyses. Even if the active principle in an allergenic material is a true protein, there is no reason to believe that other allergically inactive proteins may not be included in extracts prepared with Coca's fluid. If such inactive proteins are present, the use of protein determinations in standardizing the extracts may

lead to a confidence in the value of the chemical method which is not justified. Therefore, although precipitation with trichloracetic acid and the micro-Kjeldahl analysis of the precipitate is a very convenient method of studying the protein content of extracts of natural substances, we do not believe that the technical advantages which it possesses justify us in recommending it for standardizing substances used in the diagnosis and treatment of allergic conditions. If, in the future, further work shall prove the true protein nature of the active principle of an allergenic substance, this precipitant may develop into a useful one in the chemical standardization of such a substance.

SUMMARY

A micro-Kjeldahl method for determining the nitrogen precipitated by phosphotungstic acid is described. The method can be carried out on small amounts of material and can be completed in a relatively short period of time.

A similar micromethod for determining the protein nitrogen precipitated by trichloracetic acid is given.

Results obtained by these methods upon extracts of various substances prepared with Coca's solution are presented, and the bearing of the figures upon the complexity of such extracts is discussed.

The difficulty of basing a satisfactory chemical standardization of allergens upon such analyses is briefly considered.

CONCLUSIONS

1. The micromethods described for determining protein nitrogen are chemically accurate and economical of time and material.

2. The methods should be applicable to the standardization, for clinical purposes, of allergenic substances, whose active principle has been shown to be a true protein.

3. The use of such methods in the chemical standardization of allergenic substances less thoroughly studied is not warranted on theoretical grounds.

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IONIZATION OF THE NASAL MUCOSA. RELATIONSHIP BETWEEN REAGINS IN THE BLOOD AND EFFECT OF TREATMENT

Preliminary Report*†

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THE application of the principle of iontophoresis to the nasal mucous membranes is not new. However, apparatus for such therapy has been improved by Warwick,[1] who reported striking results in the treatment of hay fever and hyperesthetic rhinitis. During the past year, "ionization" of the nose has come to be applied somewhat extensively for local allergic disorders. The procedure consists of applying a solution of certain metallic salts to the mucous membrane by means of a nasal pack. When passing a galvanic current through this solution, ions thereby released presumably act on the surrounding tissue. In a few days a slough is discharged, and restitution of what appears to be normal mucous membrane follows. The mechanism by which a slough is formed was not investigated, although it is possible that ionization under the above circumstances is but an effective form of cautery.

The mechanism of relief under these circumstances is of interest. If symptoms before treatment are regarded to result from contact between the specific allergen and the tissue antibody, then relief may be attributed to mechanical removal of antibody. The restored mucous membrane is not sensitive so that inhaled or ingested allergens evoke no response. The procedure of ionization offers an opportunity to observe how soon and under what circumstances the newly formed epithelial tissue may become sensitive. It was surmised that if the reagin content of the blood persisted, sensitization would soon return. On the other hand, if no reagins could be demonstrated, the process of new local antibody formation would probably be slower. There are other possibilities to explain relief of symptoms, but the above only was investigated.

Patients were treated at the Washington University Out-Patient Department after a routine allergic history was taken and a careful otorhinologic examination was made by a member of the staff. This included a study of nasal secretions in which the relative number of

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†This investigation was made possible by a gift secured from an anonymous donor by Dr. Arthur M. Alden. Dr. Alden also referred several of the patients included in this report.

TABLE I

RESULTS OF SKIN TESTS AND PASSIVE TRANSFER ON 25 PATIENTS WHO WERE IONIZED

PATIENT	DIAGNOSIS	PRELIMINARY		TWO WEEKS		THREE MONTHS		REMARKS
		SKIN TEST	PASSIVE TRANSFER	SKIN TEST	PASSIVE TRANSFER	SKIN TEST	PASSIVE TRANSFER	
				No Relief				
Wi.	Hay fever	++	++	++	++	++	++	
Br.	Hay fever	+++	±	+++	++	+++	++	
McC.	Hay fever	+++	−	+++	+	+++	++	
Ste.	Hay fever	+++	++	+++	+++	+++	++	
Wei.	Hay fever	++	++	+++	+	++	+	
Sh.	Hay fever	++	+	++	+	++	+	
Bl.	Hay fever	++	+	+++	+	+++	++	
Pl.	Hay fever	++	−	+	−	+	−	
Str.	Vasomotor rhinitis	+	−	+	−	−	−	Very slight improvement at 3 months. No relief at 6 months. Bad sinus infection.
				Partial Relief				
Sc.	Hay fever	++	−	+++	++	+++	++	Symptoms delayed 10 days. About 50% improvement.
Gu.	Hay fever Asthma	+++	++	+++	+++	+++	++	Hay fever improved. Asthma persisted.
De.	Hay fever	+++	−	++	−	+++	−	Hay fever improved. Developed asthma.
Ja.	Hay fever	±	±	±	±	±	−	About 50% improvement.
Hem.	Hay fever Asthma	−	−	−	−	−	−	Some improvement.

TABLE I—CONT'D

PATIENT	DIAGNOSIS	PRELIMINARY SKIN TEST	PRELIMINARY PASSIVE TRANSFER	TWO WEEKS SKIN TEST	TWO WEEKS PASSIVE TRANSFER	THREE MONTHS SKIN TEST	THREE MONTHS PASSIVE TRANSFER	REMARKS
				Essentially Complete Relief				
Ba.	Hay fever Asthma	++	++	+++	++	+++	+	Great improvement.
Cr.	Hay fever	++	+++	++	+++	++	+	Great improvement (ionized late in season).
St.	Hay fever	+	++	±	−	±	−	Great improvement (ionized late in season).
Di.	Vasomotor rhinitis	−	−	−	−	−	−	Great improvement; same results after 6 months.
Wt.	Hay fever	−	−	−	−	−	−	Great improvement.
Ba.	Vasomotor rhinitis	−	−	−	−	−	−	Great improvement; at 6 months reported complete relief. Could not appear for tests.
Sa.	Asthma	−	−	−	−	−	−	Great improvement.
Bu.	Vasomotor rhinitis	+++	±	++	+	+++	+	Complete relief.
Ed.	Vasomotor rhinitis	−	−	−	−	−	−	Complete relief.
Way.	Hay fever	−	−	−	−	−	−	Complete relief.
Tou.	Vasomotor rhinitis	−	−	−	−	−	−	Complete relief.

\+ = 0.75 sq. cm. to 1.0 sq. cm. area of wheal.
++ = 1.0 sq. cm. to 1.5 sq. cm. area of wheal.
+++ = greater than 1.5 sq. cm. area of wheal.
Dilution of pollen extracts for direct testing = 1:10,000.
Dilution of pollen extracts for passive transfer test = 1:1,000.

eosinophiles was noted. Intradermal skin tests were made and positive reactions measured with a planimeter and recorded in square centimeters. Only decidedly positive reactions were recorded. Reagins were sought for by the usual technic of passive transfer of blood serum to normal skin. Blood eosinophilia was determined. The nose was then ionized, and reexaminations by the above procedures were recorded at the end of two weeks and at three months. In a few cases observations were made at the end of six months. In some patients topical application of allergens which had caused trouble was made to the nasal mucous membranes before and after treatment.

The series studied was quite small and is being reported at this time in answer to many inquiries concerning the rationale and effect of nasal ionization. In all, 53 patients received treatment by ionization. Of these, 23 were reexamined at the end of two weeks but failed to return at the end of three months. Five others were examined after two weeks but their three-month period has not yet expired. The remaining 25 are tabulated in Table I.

As a result of ionization, the size of the initial direct skin reaction did not change materially. The size of the passive transfer test was somewhat variable, but this may be attributed to the fact that the recipients were changed. Ward patients were used, so that a repetition of the test at three months was almost always done on a different skin which may have been more or less receptive than that upon which the first transfer had been done. Blood eosinophilia was not particularly altered, and it is interesting that eosinophiles persisted in the nasal secretions, even in those patients who were relieved. The significance of this finding is not clear. Topical applications of allergens gave variable and inconsistent response.

It is, perhaps, noteworthy that of the 9 patients who secured no relief, 8 had hay fever, and of these 7 showed demonstrable reagins. On the other hand, in the group of 11 that were relieved, including 5 patients with hay fever, in only 3 were reagins found.

SUMMARY

From these preliminary findings it would appear that in patients seeking ionization treatment for nasal allergy the chances of relief are greater if no reagins in the blood are present. Whether or not this is a probability can be confirmed only by further statistics.

REFERENCE

1. Warwick, H. L.: Laryngoscope 44: 173, 1934.

A NEW METHOD OF PREPARATION OF POLLEN EXTRACTS

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REGARDLESS of the fact that some pollen extracts will cover the treatment of hay fever, there is still considerable to be desired when the ordinary methods of preparing antigens permits the use of only three to five pollens.

In testing hay fever patients, there is frequently a reaction to 12 to 15 pollens, many not in closely related botanical groups. These pollens are frequently, if not always, left out of the antigenic extracts if the reactions are not strongly positive. This leaves the patient sensitive to these pollens, and, if badly enough exposed, he will have hay fever.

Study was done in order to find some way to improve antigenic extracts, or at least the scope of pollen therapy. A plant survey of the surrounding territory was made, and pollen slides were examined. This, in connection with the summary of skin reactions in hay fever patients, formed the basis for the preparation of pollen extracts used.

It would probably be more ideal to use a pollen mixture obtained directly from the air. This was attempted but because of mechanical difficulties the plan has been temporarily dropped. The pollens were mixed dry in a ratio similar to their occurrence in the air, abundance of growth and ability to produce skin reactions in typical patients (Table I).

In the mountain states as elsewhere, there are three pollen seasons. Box elder is the worst of the offending trees, but the list includes many others. Timothy, orchard grass, and June grass are about equally common in the early summer, while Russian thistle, western ragweed and sage and related chenopods predominate in the late summer and fall season.

Table I shows the composition of each of the three pollen mixtures. (Note that the three groups do not contain the same total quantity of pollen.) Extracts of each of the dry mixtures were made in a solution of glycerin and distilled water—50 per cent of each by volume made up to 0.86 per cent sodium chloride—the solution being slightly acid to litmus and to phenolphthalein. Alkaline solutions were not used because it was found that acid extracts gave greater skin reactions. Such extracts remain potent for many months. Dilutions used in treatment were made up from the stock mixtures with 5 per cent glucose and 0.5 per cent phenol in normal salt solution. This mixture is less irritating than glycerin solution when administered, but should be made up fresh at frequent intervals in order to insure potency.

The strength of the extract was fixed arbitrarily in terms of the principal pollen used and without regard to whatever other pollens might be in the mixture—it being assumed, according to the laws of solvents and solutions, that each goes into solution independent of the others. Thus, mixture No. 3, containing 0.5 gm. of Russian thistle pollen in 20 c.c. of extracting fluid, is a 1:40 extract of Russian thistle and is called a 1:40 extract of the whole group. From this stock, dilutions are made up to 1:40,000 as indicated.

Most patients receive first 0.01 c.c. of a 1:40,000 dilution of any extract or combination of extracts. All treatments are given intradermally. The treatments are given at intervals of one day in severe cases, to ten to fourteen days in patients having few symptoms.

TABLE I

PROPORTION OF POLLENS USED IN STANDARD MIXTURES

SPRING (NO. 1) ANTIGEN		EARLY SUMMER (NO. 2) ANTIGEN		LATE SUMMER (NO. 3) ANTIGEN	
POLLEN	GRAM	POLLEN	GRAM	POLLEN	GRAM
Box elder	0.50	Timothy	0.50	Russian thistle	0.50
Lombardi poplar	0.15	Orchard grass	0.50	Sueda Moquini	0.20
Carolina poplar	0.15	June grass	0.50	Alanrolfia	0.50
Cottonwood	0.15	Bermuda	0.10	Fr. Tenuifolia	0.50
Maple	0.10	Juncus Balticus	0.10	Fr. Acan.	0.50
Birch	0.10			Amb. Elatior	0.35
Oak	0.10			Black sage	0.25
Shadscale	0.10			Gray sage	0.25
Juncus Balticus	0.10			Kochea. Scop.	0.25
Willow	0.10			Atriplex (Annual) several each	0.25
Apricot	0.10				
Plum	0.10			Chenopods (Annual) several each	0.25
Ash	0.10				
Apple	0.10			Nettle	0.15
Dandelion	0.15			Amaranth	0.10
				Plantain	0.10
				Iva Xanth.	0.10

Kahn has shown that the skin has a reaction capacity for allergens above most other tissues. For this reason all treatments are given intradermally. The greatest volume and concentration found necessary at present are 0.05 c.c. of 1:4,000 dilution of a single or combined antigen.

One fundamental idea behind this system of treatment is that small amounts of pollen extract are adequate for treatment of hay fever. This has been proved in my own practice over a period of four years. The method has been used on 350 patients with satisfactory results in 84 per cent. Of these, 299 never received a total of more than 0.1 c.c. of 1:4,000 dilution, and 42 never received more than a total 0.1 c.c. of 1:500 dilution. Of the original 55 patients who first used this small dosage method, 24 require no further treatment. They are having no symptoms. Others require only an occasional treatment to keep them comfortable. These results are certainly satisfactory.

It is a common occurrence in questioning hay fever patients with asthma to find that after taking the regular large dosage treatment, they are made worse after each inoculation, and may even have an accompanying general reaction. This is entirely avoided by small doses. Danger of general reactions is nil, large sore arms are avoided, the patient is not afraid of the treatment, and, most important, the results are satisfactory.

By weighing, diluting, and counting pollen granules, it has been found that one pollen unit is equivalent to 80 to 100 pollen grains. From calculations made from pollen counts of the air, the average person inhales from 8 to about 1,000 pollen grains during twenty-four hours, depending on weather conditions, such as wind, rain, sunshine, and on the number of plants in bloom. Knowing this, why should a patient ever receive more pollen extract in his treatment than he absorbs through the mucous membranes? This reasoning formed the basis for small dosage treatment of hay fever.

Can harm come from treating a patient with pollen to which he may not be sensitive? The question is pertinent and difficult to answer. So far, I can only say that I have not observed bad results. It is my impression that the method has prevented some patients from becoming sensitive to pollens to which they are constantly exposed, but for which they give little or no skin reactions.

Poor results in the ordinary method of treatment may occur because the patient is sensitive to pollens which are not included in the mixture given. This is avoided if a comprehensive mixture is used.

SUMMARY

The advantages of giving small doses in the treatment of hay fever appear to be as follows: (1) They may stimulate antibody production, and (2) there is little danger of sore arms or of general reaction.

The advantages of using mixtures of pollens of local plants that cause hay fever are: (1) They resemble the pollen mixtures that the patient inhales, and (2) they are potent and capable of giving good results.

NORMERGIC AND ALLERGIC INFLAMMATION*

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THE first, ordinary or normergic response of the body to a foreign protein (pollen, vegetable protein, bacteria, foreign animal protein) that has penetrated the tissues varies from a negligible reaction to a severe inflammation, depending upon the inherent toxicity of the substance and upon the dose. The body is then more or less sensitized to the foreign material, and upon repeated exposure to it, the inflammatory response differs from the original reaction in that the onset is more rapid, the course more violent, and the resolution of the process slower. Hyperergic reactions of this sort analogous in many respects to conditioned reflexes, occur not only in the clinical allergies but also in other forms of anaphylaxis, in idiosyncrasy, and in immunity.

In contrast to the hyperergic state of allergy there is a hypoergic or anergic state in which the body fails to react to an excitant, a condition not to be confused with acquired immunity.

As is well known, allergic reactions are influenced by the number and size of the sensitizing and shocking doses and by the time intervals. They vary from functional, vascular responses and hypertonus or spasm of smooth muscle to the severe necrotizing inflammation characterizing the Arthus phenomenon. Under certain circumstances specific granulomatous lesions result. Although it is possible that all the cells in the body may be sensitized, allergic reactions are ordinarily exhibited by the arterial system, connective and lymphoid tissues, and synovial, ectodermal and entodermal membranes, whereas the parenchymatous tissues are usually spared.

Rössle, who for the past twenty years has contributed greatly to the knowledge of the tissue changes in allergy, has recently[1, 2, 3] critically analyzed the literature of the subject. Much of this paper consists in extracts of that review. As presented by Rössle, the allergic reaction may consist entirely of a focal hyperergic reaction localizing the antigenic poison as in the Arthus phenomenon; and this organ or tissue disease prevents a blood disease. If, however, the hypersensitive state is not very marked or the shocking dose is unusually large, and in spite of local blockage by hyperergic inflammation some of the allergen spills over into the circulation, the safeguarding of the individual is achieved by the spleen, liver, and other allied protective embankments. If still

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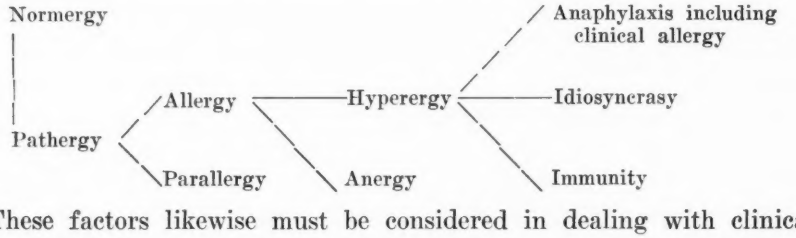
more allergen chances to get into the circulation, the physiologic anergy of the remaining endothelium changes to hyperergy, and by the arrest of living or dead poison on the intima, various vascular granulomata result. If this last safeguard is finally broken through, the allergen penetrates to the tissues and produces different sorts of inflammation depending upon the kind and degree of the hypersensitivity. The successful production of endocarditis, arthritis, and myocarditis resembling the lesions of rheumatism by repeated and attenuated doses of living or dead antigens may be explained on this basis.

Evidence is accumulating that many of the drug idiosyncrasies with reversible and irreversible tissue changes are, in reality, true hyperergic allergic reactions, the drug as such or the drug as modified by the body acting as a hapten.

The hyperergic reactions in immunity are completed without injury to the body health. They represent a perfect adaptation of the body to the various antigens.

A further complicating factor influencing certain inflammations is the reaction of specifically sensitized tissues to a nonspecific allergen. A typical example of this sort is the transitory positive tuberculin reaction in certain individuals following vaccination with cowpox virus. Moro and Keller[3] consider such reactions to be parallergic. Other parallergic reactions are likewise involved in mixed infections, superinfections and complications of infections. The severe hemorrhagic lesions experimentally produced in the Sanarelli and Shwartzman phenomena are likewise parallergic reactions, as are also the severe vascular lesions with thrombosis and gangrene produced by Marcus and Schmidt-Weyland in rabbits sensitized with certain bacteria or bacterial toxins and then injected repeatedly with adrenalin. It is possible likewise that the hemorrhagic necrotic lesions in the liver in eclampsia may result from the action of pituitary hormone in excess after sensitization of the individual by some foreign excitant.

In analyzing the tissue changes of inflammation the rôle of the following factors outlined by Rössle must be considered:



These factors likewise must be considered in dealing with clinical manifestations. The incubation period of various diseases, for instance, may represent a period during which a state of hypersensitivity is being developed to be followed by the production of characteristic lesions and

symptoms. Furthermore, persistent or increased hypersensitivity may determine the occurrence of sequelae, mixed infections, superinfections, or complications.

The milder reactions to allergen of the sensitized body may be exhibited in functional changes only, and expressed in sneezing, coughing or in the pain of spasm or colic. Among the anatomical expressions of response to repeated exposure to very small amounts of foreign protein is the hypertrophy of smooth muscle (arteries, pylorus) resulting from repeated spasm or more serious involvement of the muscle cells. The possible etiologic relationship of repeated allergic responses to certain arterial diseases suggests itself.

The evidence is quite convincing that in rheumatism, the body is in a state of low grade sensitization and that the lesions of fibrinoid degeneration of connective tissues and of Aschoff body formation are produced by repeated small doses of some foreign substance that in the normergic individual would induce no appreciable disturbance. Streptococci and their products have been implicated by Bieling,[1] Moon and Stewart[1] and others in this disease. Similar lesions however have been produced in animals by Klinge[2] with horse serum in small amounts after previous sensitization with very small doses of the same foreign protein. In addition to slight degenerative changes in the synovial membrane and other soft tissues of the injected joints a number of animals showed granulomatous lesions in the myocardium and endocardium, all resembling the changes occurring in rheumatism. In animals more highly sensitized with horse serum, reinjection in joints of a large shocking dose resulted in frank purulent arthritis.

Rössle[1] directly observed the changes of normergic and allergic inflammation in frog mesentery preparations. Application of swine serum in frogs previously sensitized to this foreign protein led to prompt stasis of the blood much greater in extent than in the untreated animals, and at the margins of the poisoned area there was a reversal of capillary blood flow. In addition there was a much more rapid and much more intense accumulation of fluid and leucocytes in the sensitized animals, a greater tendency to hemorrhage and finally a greater delay in the resolution of the process.

The inflammatory reactions associated with the well-known clinical allergies (atopies), hay fever, asthma, urticaria, vasomotor rhinitis, eczema, gastrointestinal disturbances, etc., are all characterized by considerable edema and infiltration with eosinophiles, plasma cells and other wandering cells. Involved mucous membranes also show evidence of hypertrophy of smooth muscle and active secretion of mucus. The histopathologic changes in nasal and sinus mucous membrane of 29 cases of this sort observed by us correspond to those described by Finck,[4] Hansel,[5] Weille,[6] and Coates and Ersner[7] and are remarkably alike in spite of the

dissimilarity in chemical composition of the causative pollens and dusts. Similarly our postmortem observations of the tissues in a case of bronchial asthma are in accord with the excellent descriptions of Huber and Koessler,[8] Kountz and Alexander,[9] Steinberg and Figley.[10]

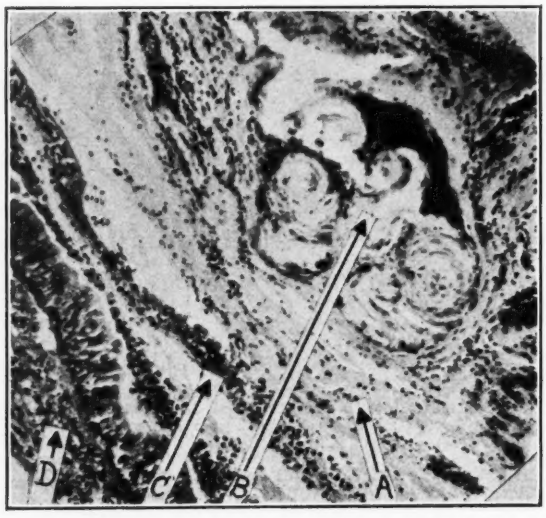


Fig. 1.—Reversible allergic inflammation. Case 3, bronchial asthma. Photomicrograph, low magnification. *A*, Mucus; *B*, Curschmann's spirals; *C*, eosinophiles, neutrophiles; *A*, *B*, and *C*, in lumen of bronchus; *D*, bronchial mucosa with edema, eosinophiles, mononuclears.

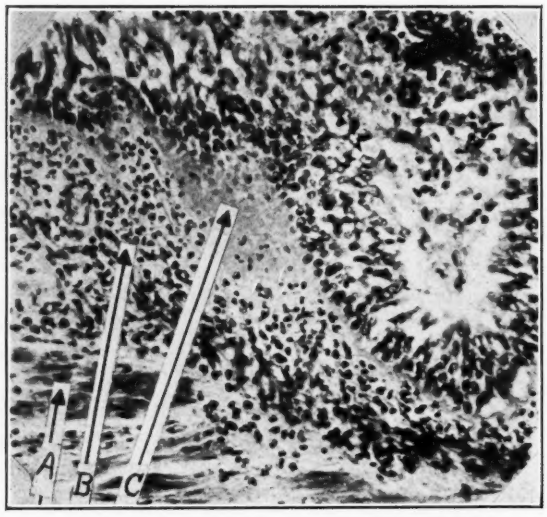


Fig. 2.—Reversible allergic inflammation. Case 3, bronchial asthma. Photomicrograph, low magnification. *A*, Thickened smooth muscle cells; *B*, eosinophiles, neutrophiles; *C*, bronchial wall with edema.

Reversible histologic changes like those described above were observed to such widely differing allergens as ragweed pollen and house dust in experimentally produced wheals in allergic individuals.[11] In that study

of 31 specimens of human skin, it was noted that from twenty to thirty-five minutes after the injection of allergen or histamine in an allergic individual there was moderate to marked regional congestion, edema and leucocytic infiltration with half or more of the wandering cells of eosino-

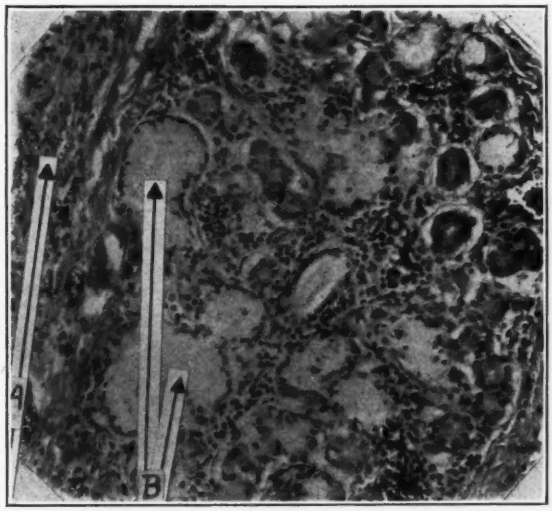


Fig. 3.—Reversible allergic inflammation. Case 3, bronchial asthma. Photomicrograph, low magnification. *A*, Edema, eosinophiles, mononuclears; *B*, distended mucous glands.

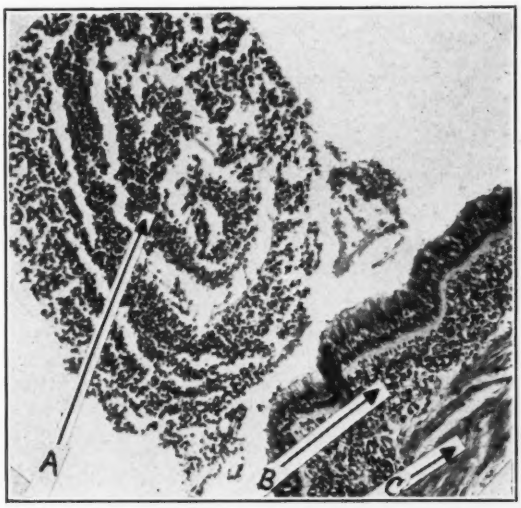


Fig. 4.—Reversible allergic inflammation. Case 4, bronchus in periarteritis nodosa. Photomicrograph, low magnification. *A*, eosinophiles, neutrophiles (lumen); *B*, bronchial wall with edema, eosinophiles, mononuclears; *C*, thickened smooth muscle.

philic type. Three hours later the inflammation was no longer characteristic, the eosinophiles represented but 10 per cent of the wandering cells, and the inflammation resembled an ordinary inflammation of considerable intensity. At the end of twenty-two hours the tissues showed a subsid-

ing inflammation of ordinary type. In nonallergic individuals the reactions were much less marked, at times negligible and unaccompanied by a transitory local eosinophilia. These findings apparently give some support to the view of Lewis[12] and others that in the allergic individual there is an increased tendency of stimulated or injured cells to release histamine or a histamine-like substance which then causes the local reaction.

Reversible inflammatory lesions suggestively allergic in nature have been observed in nearly all the organs of the body in several hundred specimens in the course of routine examination of surgical and autopsy material at Mount Sinai Hospital during the past ten years. The majority were from the appendix, gallbladder and fallopian tubes. Other tissues similarly involved were nasal, sinus and bronchial mucosa,

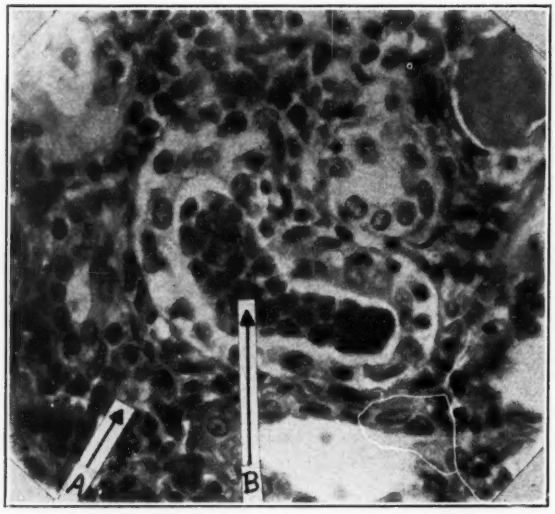


Fig. 5.—Reversible allergic inflammation. Case 4, kidney in periarteritis nodosa. Photomicrograph, high magnification. *A*, Mononuclears in interstitial tissue; *B*, cast of eosinophiles in tubule.

synovial membrane, voluntary muscle, stomach, intestine, lymph node, bone marrow, and in a few instances other organs including lung, ovary, kidney, bladder, and brain. In a number of these cases there was a definite history of clinical allergy of some sort. Typical examples in this group are the changes in the appendix and gallbladder described below.

In certain diseases such as rheumatic fever, tuberculosis, and syphilis, the allergic inflammatory tissue changes may be not only reversible (positive tuberculin reaction [skin] and slowly resolving exudative pneumonia in tuberculosis, positive luetin reaction in syphilis) but also of more lasting granulomatous type as exemplified by the Aschoff body, the early tubercle and early gumma, produced by specific agents or their products after the body has been properly sensitized.

The most severe reactions in allergy result in irreversible degenerative and necrotic tissue changes. The classical example is the Arthus phenomenon[13] produced in the rabbit by subcutaneous injection of 5 c.c. of horse serum (heated at 56° C.) after five previous similar intraperitoneal injections at intervals of from five to six days. Instead of a negligible reaction as in the untreated animal the sensitized rabbit shows a most severe progressive sterile inflammation with marked leucocytic exudation, eventuating several days later in necrosis of the involved area (blood vessels, connective tissue, and finally epidermis). The necrotic mass sloughs off on the ninth or tenth day, leaving a caseous mass in the base of the ulceration. The phenomenon has been produced experimentally not only in the rabbit but also in other laboratory animals including the guinea pig, rat and dog,[2] and not only in the skin but also in the lung, kidney, joint, heart, pericardium, brain, stomach, and liver.[16] Caseation necrosis in the tubercle and necrosis in the gumma may be considered a similar irreversible allergic reaction in tuberculosis and in syphilis respectively.

Gregoire[1] injected animals in the region of inguinal lymph nodes with foreign protein solutions and after reinjection noted edema and hyperplasia of the germ centers of follicles not only in the regional nodes but to a less extent in those elsewhere, the changes resembling the milder alterations in status lymphaticus. Usually in these cases in man observed at autopsy the lymphoid tissues (nodes, spleen, intestine) show well-marked degenerative and necrotic changes in the germ centers of the majority of follicles. The possible allergic nature of status lymphaticus has been suggested by Symmers,[14] Rössle,[1] Waldbott,[15] and others. Another necrotizing lesion that may be allergic in type is that observed involving the intima of small arteries in cases of nephrosclerosis.

Evidence is accumulating that many of the drug idiosyncrasies with reversible and irreversible tissue changes are in reality true allergic reactions and that the drug as such or the drug as modified by the body acts as a hapten. Quick[16] believes the liver necrosis in cinchophen poisoning to be the result of a true allergic reaction. He explains the absence of skin and Prausnitz and Küstner reactions by the assumption that a metabolic derivative of cinchophen rather than the drug itself acts as a hapten. A similar explanation would apply to the action of arsphenamine and other drugs.

Strongly suggesting allergic inflammation with reversible and irreversible changes are the findings in periarteritis nodosa as first pointed out by Gruber.[17] In three such cases observed at Mount Sinai Hospital, Cleveland, the arteries in various portions of the body showed in addition to the inflammatory changes observed in the well-known clinical allergies, focal areas of degeneration and necrosis and at times regional granuloma formation.

In our experience reversible and irreversible changes similar to those in periarteritis nodosa occur in other tissues especially in the appendix and gallbladder. The degenerative and necrotic changes present in a

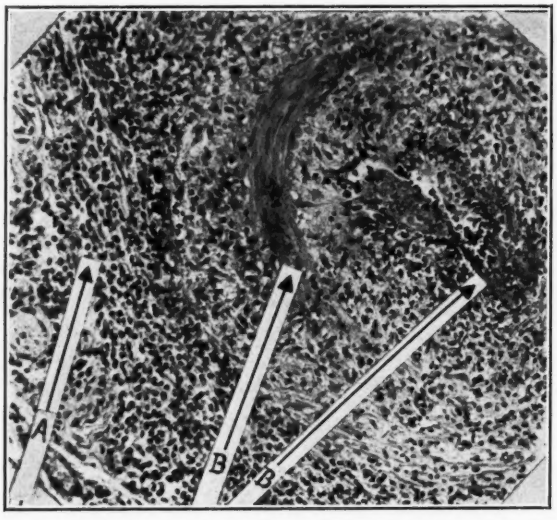


Fig. 6.—Reversible and irreversible allergic inflammation. Case 4, artery in periarteritis nodosa. Photomicrograph, low magnification. *A*, Reversible round cell infiltration (eosinophiles, mononuclears, neutrophiles); *B*, irreversible degeneration and necrosis in intima and media.

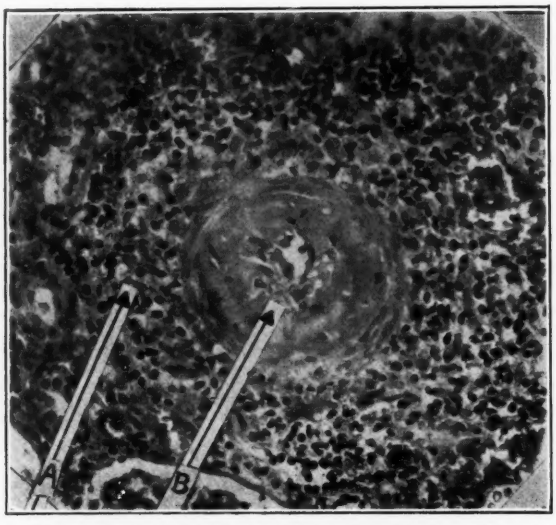


Fig. 7.—Reversible and irreversible allergic inflammation. Case 4, artery in periarteritis nodosa. Photomicrograph, low magnification. *A*, Reversible round cell infiltration (eosinophiles, mononuclears, neutrophiles); *B*, irreversible degeneration and necrosis, intima.

number of these cases as well as the edema, local eosinophilia, and hypertrophy of smooth muscle may well have been the result of an allergic reaction rather than of direct bacterial action.

In addition to rheumatism and tuberculosis, according to Rössle[2] the following diseases are claimed to be of allergic nature: croupous pneumonia (Lauche); periarteritis nodosa (Gruber, Nieberle, Tschili-

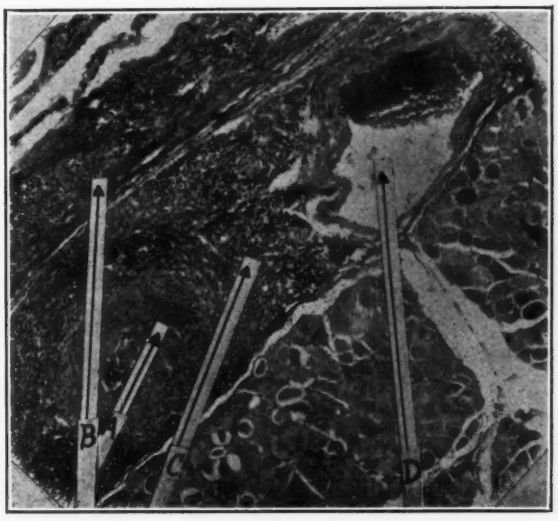


Fig. 8.—Reversible and irreversible allergic inflammation. Case 4, muscle in periarteritis nodosa. Photomicrograph, low magnification. *A*, Degeneration, necrosis (irreversible) artery; *B*, eosinophiles, mononuclears in muscle (reversible); *C*, eosinophiles, mononuclears periarterial; *D*, vein, no abnormalities.

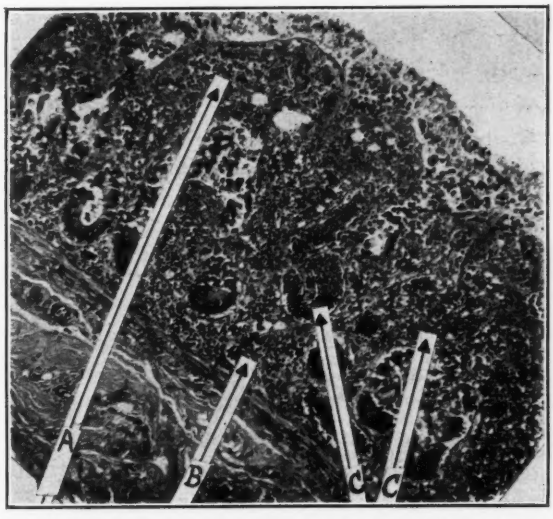


Fig. 9.—Reversible and irreversible allergic inflammation. Case 4, intestine in periarteritis nodosa. Photomicrograph, low magnification. *A*, Degeneration, necrosis (irreversible); *B*, eosinophiles, mononuclears, neutrophiles (reversible); *C*, congestion and hemorrhage.

kin); scrofula (Kruse); nephritis (Volhard, Luken, Kollert, Hepler-Simonds); arteritis of renal vessels in malignant sclerosis (Fahr); sympathetic ophthalmia (Riehm, Marchesan). It is well established for

eczema (Bloch, Moro). For arteriosclerosis, early rheumatoid changes are often described; and for the advanced arteriosclerosis, above all the nodular and scar forming type, it is established that it arises through scar forming rheumatic arteritis (Wiesel, von Glahn and Pappenheimer, Chiari, Klinge). In typhoid fever there are many participating manifestations that remind one of allergic tissue reactions, also in undulant fever (W. Löffler and A. v. Albertini). Bieling believes the same for Bang's disease with its joint affections.

SUMMARY

The first, ordinary or normergic response of the body to a foreign protein (pollen, vegetable protein, bacteria, foreign animal protein) that has penetrated the body tissues varies from a negligible reaction to a severe inflammation depending upon the inherent toxicity of the substance and upon the dose. The body is then more or less sensitized to the foreign substance, and upon repeated exposure to it the inflammatory response differs from the original reaction in that the onset is more rapid, the course more violent, and the resolution of the process slower. Hyperergic reactions of this sort analogous in many respects to conditioned reflexes occur not only in the clinical allergies but also in other forms of anaphylaxis, in idiosyncrasy, and in immunity.

In contrast to the hyperergic state of allergy there is a hypoergic or anergic state in which the body fails to react to an excitant, a condition not to be confused with acquired immunity.

As is well known, allergic reactions are influenced by the number and size of the sensitizing and shocking doses and by the time intervals. They vary from functional, vascular responses and hypertonus or spasm of smooth muscle to the severe necrotizing inflammation characterizing the Arthus phenomenon. Under certain circumstances specific granulomatous lesions result. Although it is possible that all the cells in the body may be sensitized, allergic reactions are ordinarily exhibited by the arterial system, connective and lymphoid tissues, and synovial, ectodermal and entodermal membranes, whereas the parenchymatous tissues are usually spared.

Evidence is accumulating that many of the drug idiosyncrasies with reversible and irreversible tissue changes are in reality true hyperergic allergic reactions, the drug as such or the drug as modified by the body acting as a hapten.

The hyperergic reactions in immunity are completed without injury to the body health. They represent a perfect adaptation of the body to the various antigens.

A further complicating factor influencing certain inflammations is the reaction of specifically sensitized tissues to a nonspecific allergen. A typical example of this sort is the transitory positive tuberculin reaction

in certain individuals following vaccination with cowpox virus. Moro and Keller consider such reactions to be parallergic. Other parallergic reactions are likewise involved in mixed infections, superinfections, and complications of infections. The severe hemorrhagic lesions experimentally produced in the Sanarelli and Shwartzman phenomena are likewise parallergic reactions, as are also the severe vascular lesions with thrombosis and gangrene produced by Marcus and Schmidt-Weyland in rabbits sensitized with certain bacteria or bacterial toxins and then injected repeatedly with adrenalin. It is possible likewise that the hemorrhagic necrotic lesions in the liver in eclampsia may result from the action of pituitary hormone in excess after sensitization of the individual by some foreign excitant.

In analyzing the tissue changes of inflammation the rôle of the factors outlined by Rössle and appearing above in diagrammatic form must be considered.

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CASES OF REVERSIBLE AND IRREVERSIBLE ALLERGIC INFLAMMATION*

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A CASE of allergic inflammation of the nasal mucosa of reversible type and one of similar involvement of the mucous membrane of the maxillary sinus representative of 29 cases observed at Mount Sinai Hospital of Cleveland in the past ten years are presented, as is also one case of bronchial asthma studied postmortem. Similar reversible inflammatory lesions suggestively allergic in nature have been observed in nearly all the organs of the body in several hundred specimens in the course of routine examination of surgical and autopsy material during the past ten years. The majority were from the appendix, gallbladder, and fallopian tubes. Other tissues similarly involved were synovial membrane, voluntary muscle, stomach, intestine, lymph node, bone marrow, and in a few instances lung, ovary, kidney, bladder, and brain. In a number of these cases there was a definite history of clinical allergy.

Three cases of periarteritis nodosa are also presented. In these three cases the picture suggests reactions to the same allergens as cause the usual clinical allergies. The lesions which involve arterial walls particularly are apparently due to allergic reactions in a highly sensitized body and are much more severe than those of the usual clinical allergies. The histologic changes in places are of the reversible type, but in many areas the tissues show marked irreversible degenerative and necrotic changes. The wide distribution and the severity of the lesions in the autopsied case are remarkable. Furthermore, reversible and irreversible changes similar to those in periarteritis nodosa have been observed in other tissues of the body in our material, especially in the appendix and gallbladder.

CASES OF REVERSIBLE ALLERGIC INFLAMMATION

CASE 1.—B. H., white, female, married, aged thirty-two years. This is a typical case of allergic rhinitis with a blood eosinophilia of 4 per cent. Microscopic examination of the tissue removed from the nose shows typical inflammatory polyps about 7 mm. in diameter, the stroma edematous, considerably infiltrated by eosinophilic polymorphonuclears and plasma cells. Some of the mucous glands are considerably dilated. Some toward the surface contain not only mucus but wandering cells as well.

*From the Laboratory Department, Mount Sinai Hospital.
Read before the Twelfth Annual Meeting of the Association for the Study of Allergy, Cleveland, June 12, 1934.

In the stroma of the polyps there are areas of recent hemorrhage. The blood vessels of the stroma show comparatively few leucocytes within them. There are also present two masses of mucus with enmeshed wandering cells, polymorphonuclear leucocytes predominating. The majority are not eosinophilic. Many of the leucocytes show degenerative changes. (Fig. 1.) The final diagnosis was "inflammatory nasal polyps (apparently allergic inflammation)."

The inflammatory edema and the distention of mucous glands in the nasal tissue of this case although reversible have persisted and even increased to form the polyps, probably because of repeated reactions to allergen occurring at intervals frequent enough to prevent complete reversal each time of the inflammatory process.

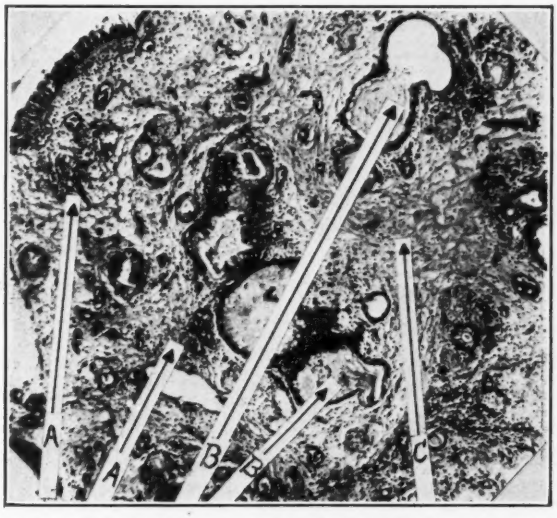

Fig. 1.—Reversible allergic inflammation. Case 1, nasal polyp. Photomicrograph, low magnification. *A*, Eosinophiles, mononuclears; *B*, distended mucous glands; *C*, edema.

CASE 2.—A. S., white, male, single, aged fifty years. This is a typical case of allergic sinusitis of thirty-seven years' duration, with a blood eosinophilia of 10 per cent.

Microscopic examination of tissue removed from the antrum shows four polypoid masses, the largest approximately 1 cm. long diameter. The masses are covered along one surface by columnar epithelium, with many goblet cells present. In places, the surface epithelium is denuded. The underlying loose areolar tissue shows marked edema, and a rich cellular infiltration by wandering cells with eosinophiles greatly predominating. The mucous glands are dilated and contain abundant lavender-stained material. In places there are focal areas of infiltration by neutrophilic polymorphonuclear leucocytes. Blood vessels show infiltration of the walls and margination by wandering cells. (Fig. 2.) The final diagnosis

was "subacute sinusitis with edema and polyp formation (apparently allergic inflammation)."

The lesion in this case developed apparently in much the same way as did the nasal polyps in the first case.

CASE 3.—F. G., white, male, aged sixty years. This is a case of bronchial asthma and is unusual in that the patient had had only one previous attack nine weeks before the terminal illness. The evidence in this case pointed to an intrinsic factor. During the final illness the patient was in a continuous asthmatic state and expired while having a severe attack of dyspnea.

At autopsy the lungs showed the most significant changes. They were much more voluminous than average and entirely filled the pleural sacs,

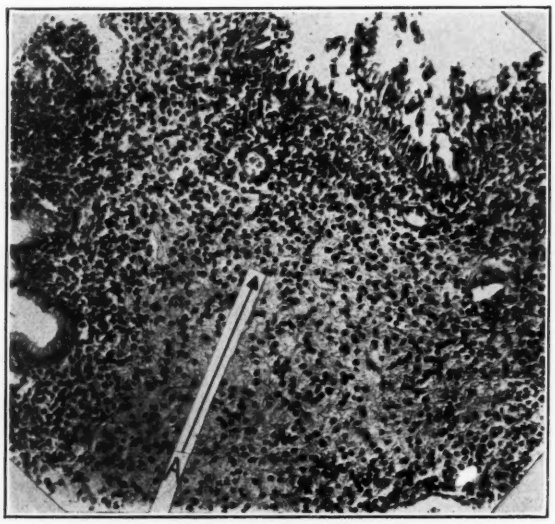

Fig. 2.—Reversible allergic inflammation. Case 2, polypoid sinusitis. Photomicrograph, low magnification. *A*, Edema, marked round cell infiltration (90 per cent eosinophiles).

having pushed the diaphragm lower than average. All lobes were dark red, meaty in consistence, apparently everywhere air-containing, however. The bronchioles showed thickened walls and stood out prominently on cut surface. Bronchi and bronchioles contained abundant tenacious greenish yellow viscid material. Cut surfaces of lungs were dry, and no emphysematous blebs were present.

Microscopic examination of the lung sections showed the lumina of bronchi filled with mucus in which there were numerous neutrophilic polymorphonuclears, eosinophiles, and mononuclear cells. The cells of the exudate were most abundant immediately overlying the mucosa. There were also characteristic Curschmann's spirals present. The walls of the bronchi showed the smooth muscle cells thickened; in places the muscle was edematous and infiltrated by wandering cells. The mucosa was

thickened by edema and richly infiltrated by eosinophiles, neutrophilic polymorphonuclears, and mononuclear cells. The blood vessels in the submucosa were dilated and engorged. The mucous glands in the walls of the bronchi were greatly distended with mucus; the cells contained abundant mucus; the nuclei were pushed to the periphery (Figs. 1, 2, and 3 of preceding article). Many bronchioles were filled with neutrophilic polymorphonuclears. The alveoli about involved bronchioles contained abundant exudate in which there were numerous neutrophilic polymorphonuclears and red blood cells. In places there were bacterial masses in the exudate; in places there were refractile bodies in the bronchial lumina and mucosa, suggesting pollen granules. The changes were most marked in the submucosa of the bronchi with considerable edema and wheal-like swellings between mucosa and muscularis, the mucosa showing prominent folds. There were areas of bronchopneumonia and, in places, areas of hemorrhage.

Sections showed gram-positive and -negative bacterial masses in the exudate, gram-positive cocci predominating.

Section of trachea showed considerable edema of submucosa, moderate infiltration of all coats by lymphocytes and eosinophiles.

Positive findings in addition to the changes in the lung, were degenerative and necrotic changes in the central portions of some of the follicles of the hilic lymph nodes and spleen. The brain showed some edema of the meninges over the convexities and areas of serous meningitis.

The bronchial mucosa in this case showed reversible changes similar to those in the upper respiratory mucosa in the first two cases. In addition the smooth muscle cells in the walls of bronchi appeared definitely thicker than average.

CASES OF REVERSIBLE AND IRREVERSIBLE INFLAMMATION

The histologic changes described in the above cases are representative of the lesions in the ordinary clinical allergies. Similar lesions associated in many places with irreversible, severe, degenerative and necrotic changes in the fixed tissue have been observed at Mount Sinai Hospital in three cases of periarteritis nodosa. This disease is now considered by many to be of allergic nature, and the histologic changes in the cases presented below strongly suggest reactions in highly sensitized individuals to the same allergens that cause the usual clinical allergies (pollens, vegetable proteins, and animal proteins). In other recorded cases bacteria have been considered the causative agents.

Lindberg[1] reviewed the literature comprising 150 cases of periarteritis nodosa to 1931 and noted the following clinical signs and symptoms: (1) fever, splenomegaly, leucocytosis, severe anemia, marked emacia-

tion; (2) polyneuritis and polymyositis in one-third to two-thirds of the cases; (3) renal symptoms; (4) alimentary canal symptoms (epigastric pain, vomiting, diarrhea).

CASE 4.—I. M., white, female, aged twenty-five years. Patient was first seen at Mount Sinai Hospital on Sept. 23, 1931, with the complaints of asthma of two years' duration and a skin rash. The rash itched considerably and recurred at twenty-four-hour intervals for some time. The patient had frequent attacks of coughing and expectoration of viscid white sputum chiefly during the winter. She had lost forty pounds in weight in the past two years. Her grandmother, aunts, and uncles all had asthma. The patient had a tapeworm infestation six years before.

Examination showed an underdeveloped and undernourished individual with a very extensive purpuric macular rash on both lower limbs and upper extremities. There was some edema of the ankles. The spleen was not palpable. Sputum showed many eosinophiles, no Charcot-Leyden crystals or Curschmann's spirals. Red blood cell count 3,000,000; hemoglobin 65 per cent; white blood cells 12,000; eosinophiles 30 per cent. Impressions on first admission were: asthma, allergic skin condition, purpura, possible tuberculosis, Henoch's purpura.

The patient was referred to the allergy clinic on October 2. She gave reactions to carrot, celery, lamb's quarter, house dust, maple and sorrel pollen. She reentered the hospital on November 10 with the complaint of pain and weakness of wrist and ankles. The pain was worse at night. There was swelling of the joints, beginning in the right ankle, increasing weakness and muscular atrophy. She also developed epigastric pain after meals, diarrhea, and further loss of weight.

Examination then revealed puffy face, pigmented skin with purpuric spots on legs. There was evidence of wrist drop on both sides, more marked on the left, with diminished reflexes and sensation and muscular atrophy of all extremities. Examination of the chest was essentially negative. The spleen was not palpable. The impression was peripheral neuritis.

Because of the possibility of trichiniasis a biopsy of muscle from the leg was done, and the microscopic description of the specimen was as follows:

Sections showed marked degenerative and necrotic changes in the walls of arteries with some nuclear fragmentation. There was marked subacute inflammation about blood vessel walls, abundant infiltration there by lymphocytes, monocytes, moderate number of eosinophiles, and neutrophilic polymorphonuclears. Voluntary muscle fibers were in part intact with striations well defined; in other areas there were degenerative changes; striations were blurred; vacuoles were present in cytoplasm in places. There was some infiltration between fibers by mononuclears and eosinophiles. The changes were most marked, however, about

blood vessels. Arterioles were everywhere thickened, with the lumina small. No trichinae were seen. Sections stained by Gram's method showed no organisms. The final diagnosis was "subacute myositis with necrotizing arteriolitis."

A small nodule in the abdominal wall was removed. The tissue showed changes similar to those in the first biopsy. A leucocytosis of 12,000 to 18,900 and an eosinophilia from 6 per cent to 28 per cent were present. There was also a moderate anemia.

During her stay in the hospital the patient had mild intermittent fever, usually less than 101° F. She became progressively weaker and had several attacks of asthma. The respiratory distress increased and death occurred on Jan. 18, 1932.

Autopsy revealed innumerable severe inflammatory lesions involving the arteries in practically all the organs and tissues of the body. In addition to edema and cellular infiltration of the wall most marked in the adventitia, many arteries showed severe degenerative and necrotic changes involving one or more coats. The lesions were characteristic of periarteritis nodosa. In places parenchymatous organs and voluntary muscles showed areas of inflammation.

The most severe arterial lesions were present in the voluntary muscles, liver, gallbladder, heart, lung, alimentary canal and kidneys. (See Figs. 5 and 9 of preceding article.) The spleen, pancreas, adrenals, bladder and internal genitalia showed less severe arterial lesions than the other organs.

The apparently reversible and irreversible changes in the following arteries are representative of the lesions all over the body:

1. Voluntary muscle (diaphragm, anterior abdominal wall, thigh, and pectoral muscle). Many arteries showed striking lesions with severe degenerative and necrotic changes involving all coats in places, and associated with marked infiltration of the walls and regional tissues by mononuclear cells, eosinophiles, and neutrophiles with eosinophiles in places predominating. In some vessels there were recent thrombi; many showed some stenosis of the lumen. The veins were uninvolved. In many places voluntary muscle fibers showed degenerative or atrophic change; in places the fibers were apparently hyalinized, and between some involved fibers there was infiltration by mononuclear cells, eosinophiles, and neutrophilic polymorphonuclears. (See Fig. 8, preceding article.)

2. Branches of coronary arteries in the epicardium showed marked degenerative changes, and marked infiltration of all coats by mononuclear cells and eosinophiles. A moderate number of neutrophilic polymorphonuclears was present. In places the adventitia of arteries was necrotic, amorphous pink stained, with numerous giant cells of foreign body type present. In places inflammatory changes involving all coats had produced stenosis or complete obliteration of the lumen. There was con-

siderable infiltration of fibrofatty tissue about blood vessels by cells of the exudate. (See Fig. 6, preceding article.) The arterioles as well as the larger branches of the coronary arteries showed inflammatory and degenerative changes of all coats.

3. Liver. Large vessels in the region of the hilum showed markedly thickened walls. There were degenerative changes in the media, proliferative changes in the intima, moderate infiltration of outer coats especially by mononuclear cells and eosinophiles. The lumina were small.

4. Gallbladder. The arteries showed striking changes. There were marked degenerative changes in the walls in places with considerable infiltration of all coats and regional tissues by mononuclears and eosinophiles, few neutrophilic polymorphonuclears. (See Fig. 7, preceding article.)

In the lung the arterial lesions were similar to those described above, and in addition many of the lobular septa showed striking edema, and infiltration by mononuclear cells, eosinophiles, and polymorphonuclear neutrophiles. In addition the bronchial mucosa showed changes characteristic of bronchial asthma with edema, infiltration by eosinophiles, and plasma cells. The smooth muscle cells in places were definitely thickened. Some glands were distended with mucus. In the lumen of many bronchi there were abundant mucus and wandering cells with eosinophiles predominating. (See Fig. 4, preceding article.)

Case 5.—W. K., white, male, widower, aged fifty-one years. Patient was first admitted to Mount Sinai Hospital on Jan. 16, 1927, with the complaint of constant shooting pains in the legs for the past two weeks. He was very weak and could walk only a few steps and then became very tired. He had chills and sweats during both the day and the night with occasional fever. He had had a nonproductive cough for four months prior to admission. He had been short of breath for the past three weeks, had had palpitation of the heart and a sense of oppression and pain in the chest. There had been occasional epigastric pain after meals, accompanied by nausea.

Patient had had infrequent headaches. Vision had been poor, and hearing not so good as average. There had been nasal discharge for some time. He had tapeworm infestation at nineteen years of age.

On examination the patient was debilitated, pale, feverish, and showed apparent gigantism. Inconstant râles were heard at the base of the lungs. Spleen was not palpable. There was hypesthesia on the anterior surface of the right thigh with hyperesthesia posteriorly. A generalized adenopathy was present. A leucocytosis, and eosinophilia averaging 34 per cent were present.

Biopsy of voluntary muscle showed considerable leucocytic infiltration of the intima especially, somewhat less marked in the adventitia and media. Many of the leucocytes were eosinophilic. In a number of places there were small old hemorrhages. Some altered blood appeared

within the monocytes. In some of the arteries there was granulation tissue partially occluding the lumen. In places in the granulation tissue there was foreign body giant cell formation. No larvae or parasitic ova were observed anywhere in the sections.

Sections stained by Gram's method showed no bacteria. Veins showed no appreciable abnormalities. The final diagnosis was "subacute arteritis and myositis (periarteritis nodosa)."

Biopsy of a large lymph node showed on microscopic examination of several sections sinuses distended with monocytes and eosinophilic leucocytes, especially, together with numerous red blood cells. In places young connective tissue was present, and there were amorphous pink stained strands especially about small thickened blood vessels. In one portion about the node there were a number of medium sized arteries

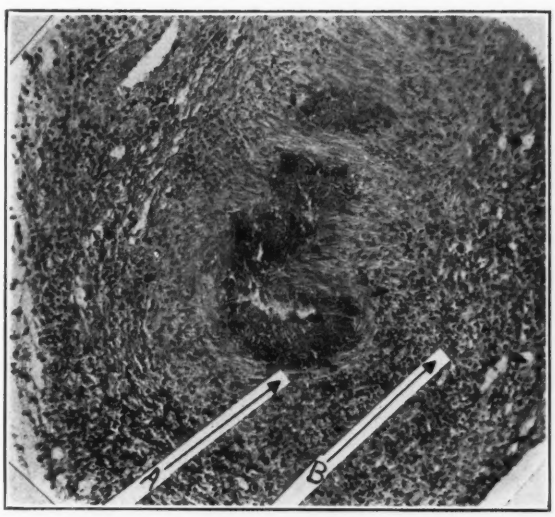

Fig. 3.—Reversible and irreversible allergic inflammation. Case 5, artery in periarteritis nodosa. Photomicrograph, low magnification. *A*, Degeneration, necrosis (irreversible); *B*, eosinophiles, mononuclears (reversible).

showing striking infiltration of walls by leucocytes, eosinophiles in places predominating. The veins showed no appreciable abnormalities. The vascular lesion was similar to that observed in the muscle. In many places the lesion was most marked in the adventitia. Refractile bodies associated with the leucocytic infiltration within and about the vessel walls were seen. Some of these were circular in outline, about 12 micra in diameter, others were rod shaped. About a number of involved blood vessels there were small old hemorrhages (Fig. 3). The final diagnosis was, "subacute lymphadenitis, subacute arteritis (periarteritis nodosa)."

CASE 6.—L. N. H., white, male, aged thirty-three years. The patient was first admitted to the hospital on May 9, 1934, with a complaint of

shortness of breath. Present illness dated back to approximately one year before when patient developed a cough and expectorated sputum. The condition then was diagnosed as pulmonary spirochetosis, and the patient was treated with salvarsan. After recovery, he was in good health with the exception of several attacks of asthma, which he had had for many years. At approximately the first of the year he had had a severe conjunctivitis which was diagnosed as phlyctenular conjunctivitis. At that time he was thought to have retinal tuberculosis and was given tuberculin injections. With these came a progressive weakening, and after eleven injections the patient was unable even to sit up. When he later started to gain strength, he began experiencing attacks of severe pain in the abdomen. These continued and became worse usually just before the patient had a bowel movement. He then began having pains about the joints with swelling. This later was accompanied by purpuric spots scattered over the body and by some hemorrhages under the nails. About six weeks prior to admission he suddenly developed shortness of breath and marked generalized edema. The past history is interesting in that he had chronic antrum disease and asthma for many years. Occasionally he had vertigo, with colored and blurred vision. Physical examination revealed a well-developed and well-nourished male of thirty-three years, slightly cyanotic. Face was pale; lips, hands and finger nails were definitely blue. Lymph nodes were palpable in the axillary, epitrochlear, and inguinal areas. Breath sounds, fremitus, and resonance were diminished at the left base. Occasionally a sibilant râle was heard at both bases posteriorly. Heart sounds were good; gallop rhythm was present. Left border extended two fingers outside the midclavicular line with the right border 1 cm. outside the side sternal line. No murmurs were audible. Blood pressure was 188/100 mm. There were a globular fullness suggestive of fluid and a definite fluid wave in the abdomen. The liver was enlarged to three fingers below the costal margin. The spleen was barely palpable. The extremities showed marked pitting edema of the ankles and shins. Pitting edema over sacrum and lower back also was present.

The clinical impression was malignant hypertension; cardiac failure, right and left; Hodgkin's disease; eosinophilic leucemia; periarteritis nodosa (?).

Laboratory reports showed a leucocytosis from 12,700 to 25,100. A blood eosinophilia from 2 per cent to 8 per cent, and a blood nonprotein nitrogen of 40 mg. per cent.

During the stay in the hospital the patient's temperature was about 100°.

Microscopic examination of a biopsy of the sartorius muscle showed striking lesions in the arteries but no appreciable involvement of the veins. The changes in the arteries involved the outer coats and regional fibrofatty tissue. In places there was not only striking round cell in-

filtration in which there were numerous eosinophiles but also degenerative and necrotic changes of some of the cells in the outer coats; and in a few of the sections areas of hemorrhage, blood clot formation, and regional miliary nodule formation appeared. Numerous eosinophilic wandering cells occurred in the nodule. The picture was typical of periarteritis nodosa. The voluntary muscle itself in a number of places showed increase in sarcolemma nuclei with many of the cells thinner than average. In a few places there was marked atrophic change, and some round cell infiltration. On further inspection one small artery showed a thrombus in the lumen, and periarterial round cell infiltration. The picture in the involved arteries was that of allergic inflammation (Fig. 4).

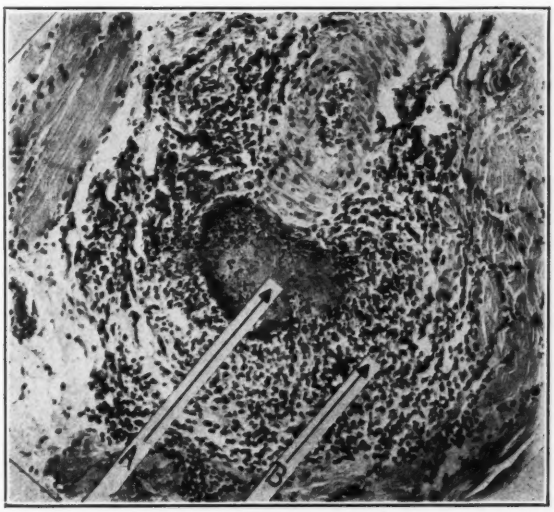

Fig. 4.—Reversible and irreversible allergic inflammation. Case 6, artery in periarteritis nodosa. Photomicrograph, low magnification. *A*, Degeneration, necrosis, hemorrhage (irreversible); *B*, eosinophiles, mononuclears (reversible).

The final diagnosis was periarteritis nodosa, subacute myositis. The patient left the hospital May 20, 1934, slightly improved.

Reversible and irreversible changes similar to those in periarteritis nodosa have been observed in other tissues of the body in our material, especially in the appendix and gallbladder. The degenerative and necrotic changes present in a number of these cases as well as the edema, local eosinophilia, and hypertrophy of smooth muscle may well have been the result of an allergic reaction rather than of direct bacterial action. Representative cases of probable allergic appendicitis and cholecystitis follow.

Case 7.—B. H., white, female, aged sixty-two years. A typical case of acute appendicitis. The patient had had hay fever many years ago, and had a son who suffered from it at present.

Microscopic examination of the gangrenous appendix showed it to be about 1 cm. in diameter, lumen several millimeters in diameter, poorly defined, irregular in outline, and continued in places through all coats. There was marked pus cell infiltration, especially of the mucosa with prominent areas of suppuration involving all coats in places. In one place the picture suggested diverticulitis. The picture was that of early rupture of all coats. There was abundant fibrinopurulent exudate over

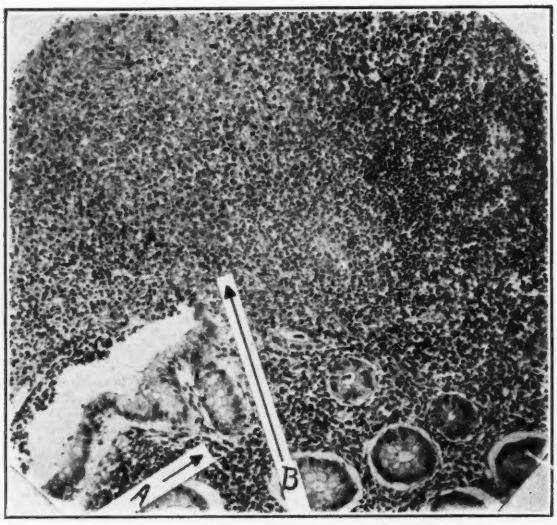

Fig. 5.—Reversible and irreversible inflammation (allergic?). Case 7, appendicitis with rupture. Photomicrograph, low magnification. *A*, Mononuclears, eosinophiles, neutrophiles (reversible); *B*, degeneration, necrosis (irreversible).

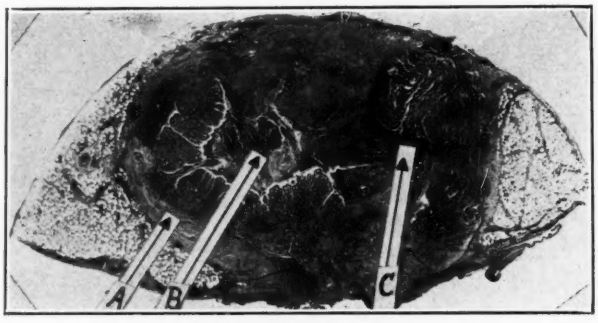

Fig. 6.—Reversible and irreversible inflammation (allergic?). Case 7, appendicitis with rupture. Photomicrograph, very low magnification. *A*, Edema, round cell infiltration (reversible); *B*, mucus in lumen; *C*, degeneration, necrosis (irreversible).

the regional serosa. In places in addition to suppuration there was necrosis with bacterial masses present, and fairly numerous eosinophiles in all coats.

Two additional smaller sections, showed less marked inflammation. One showed considerable involvement of the serosa. In these two sections, there were numerous eosinophiles in all coats. This was especially

true of the section showing involvement of serosa. The picture suggested allergic inflammation (Figs. 5 and 6).

CASE 8.—D. T., white, married, female, aged thirty-eight years. Patient was a typical case of chronic cholecystitis with gastric disturbances and belching.

Microscopic examination of the gallbladder showed considerable scarring and round cell infiltration, especially of outer coats, and areas of necrosis of mucosa and regional submucosa. In places there was necrosis of the muscle. In other places, the epithelium of the mucosa was completely necrotic. Elsewhere the cells were swollen. In the middle coats, there were innumerable eosinophilic wandering cells, the picture suggesting allergic inflammation. In the serosa, there were abun-

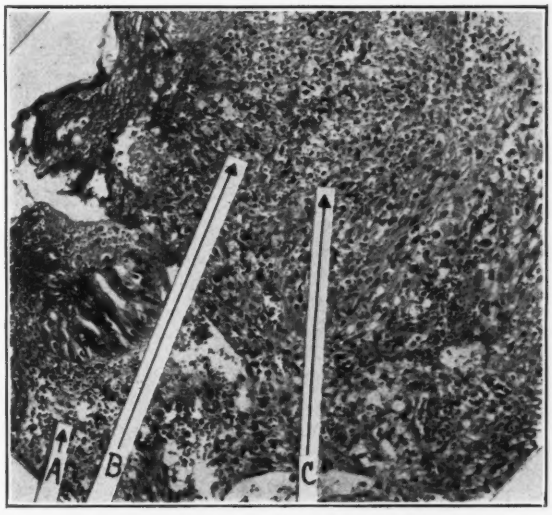

Fig. 7.—Reversible and irreversible inflammation (allergic?). Case 8, subacute and acute cholecystitis. Photomicrograph, low magnification. *A*, Hemorrhage; *B*, degeneration, necrosis (irreversible); *C*, eosinophiles, mononuclears (reversible).

dant edema, fibrin deposit, fibroblasts and capillary proliferation, and areas of hemorrhage toward the surface with some blood and fibrin clot overlying. The picture suggested allergic inflammation (Fig. 7).

The lesions in all these cases were presumably produced by foreign proteins of varying chemical composition. The lesions in each group are similar and at times apparently identical. Evidently the state of sensitization of the body and the shocking doses are of greater importance in determining the histologic changes than is the chemical composition of the causative foreign proteins.

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DISCUSSION OF "NORMERGERIC AND ALLERGIC INFLAMMATION," AND "CASES OF REVERSIBLE AND IRREVERSIBLE ALLERGIC INFLAMMATION," BY DRS. KLINE AND YOUNG

DR. MILTON B. COHEN, Cleveland.—There is practically nothing I can add to what Dr. Kline has said. I wish merely to call attention to the fact that he has spent a great deal of time and energy on this thing, and there can be no doubt at all as to the correctness of his point of view with regard to all of those things that fall into the clinical allergies; and perhaps he may ultimately be able to give us definite information as to whether some of these para-allergies really have a good, firm foundation or whether they may remain more or less hypothetical.

DR. A. F. COCA, New York.—It seems as though this characteristic histologic picture that has been so well illustrated, could be applied either to the constitutional state of the individual or to the local mechanism of the reaction; in other words, it could be taken to show that the individual was allergic or that the reaction is allergic, that is, the lesion itself. It seems as though we ought to pay some attention to this distinction.

What I want to know is whether a constitutionally allergic person reacts to a nonspecific irritant in this characteristic way with this characteristic histologic picture, and also whether a normal person who has been locally sensitized with the allergic antibodies will react in this same way; in other words, is the characteristic picture of the allergic reaction exhibited in a normal person, or only in the allergic person?

In that case, then, we should have to consider whether we can determine whether a particular lesion is allergic by this picture or whether it is occurring only in an allergic person.

There is one illustration that Dr. Kline showed us of a reaction to histamine in an allergic person. This is evidently a nonspecific reaction, yet it shows the character that he has taken to be that of allergy.

DR. MARION B. SULZBERGER, New York.—We are naturally glad to see that the pathologists are becoming so wide-awake to the possibility of the many and varied allergic or sensitization reactions in different organs. I think all of us have either surmised or hypothecated, or in some cases perhaps even proved, such reactions in various shock tissues.

As Dr. Coca said, the question of diagnosing an allergic reaction in a certain site simply on morphologic grounds, is one which has to be further scrutinized. I doubt whether we can accept a certain morphology alone as an absolute proof that a lesion is caused by an allergic process, or as proof that that lesion occurs in a constitutionally allergic individual.

For instance, the experiments of Dr. Kline and Dr. Cohen seem to me somewhat in contradiction with the similar experiments of M. Walzer and of W. Jadassohn with drugs and with allergens in normal and hypersensitive individuals. Jadassohn found that the injection of histamine in a normal individual would produce a wheal which, when excised, could not be reproduced by a hypersensitive wheal produced by an allergen in a hypersensitive individual.

In my few attempts I have found that eosinophiles can be found in the noses of many seemingly nonallergic individuals when the mucous membrane has been irritated to a certain degree.

We all know the varied histologic and morphologic reactions to the tubercle bacillus, to the treponema, and to fungi. All these are proved to be determined, to a great extent, by the allergic state of the tissue.

I am decidedly of the opinion that there can be no sure morphologic diagnosis of allergy, for there is no histology which can be considered pathognomonic; and, further, I think that it has long been known that there is practically no organ which is immune to allergic reaction; and no form of inflammatory response, from the most rapid and exudative to the most chronic and destructive, which may not be encountered in a reaction due to sensitization.

DR. GEORGE L. WALDBOTT, Detroit.—Among the cases of status thymicolymphaticus which I have had occasion to study, there is a certain group in which positive evidence of allergy, such as a family history, eosinophilia, etc., is found. In another group of cases, however, such evidence is lacking. This suggests that there may be two types of allergy, one with an allergic background, and one, as Drs. Kline and Young pointed out, in which an apparently normal state existed previous to the onset of the disease.

I think the same condition prevails in urticaria. We see urticaria in connection with serum disease which occurs in nonallergic individuals. This urticaria is entirely different from urticaria which accompanies allergic shock, and which is definitely an allergic manifestation. I wonder whether the authors would be able to say anything with reference to this question, particularly whether or not they had an opportunity to study the histology of the wheal in serum sickness as compared with that of allergic shock.

I should also like to ask the authors whether they have any information concerning the rôle of lymphocytes in allergic inflammation, particularly whether or not lymphocytes in allergic inflammation are more prevalent than in nonallergic inflammation.

Another question arises with the authors' mention of an Arthus phenomenon in the appendix. I believe an analogous condition occurs in the lungs. I have recently had opportunity to observe patients in allergic shock who had pneumonia within six to eight hours after an injection of serum was given. I feel that in these lesions a "noninfectious pneumonia" is based on an Arthus phenomenon in the lungs similar to that described by the authors in the appendix.

DR. ISIDOR HARRISON TUMPEER, Chicago.—I should like to add a clinical fact which may help to answer the question whether the tissue changes depend on whether the individual is allergic or normergic.

I have clinical observations which have been recited but not published to indicate that the allergic child is prone to react in a hyperergic or exaggerated manner to infections, such as pneumonia, varicella, and other infectious diseases.

DR. RALPH H. SPANGLER, Philadelphia.—I should like to ask Dr. Kline whether a blood eosinophilia was present in any of the patients whose lesions showed the presence of the marked increase of eosinophile cells.

DR. HARRY S. BERNTON, Washington, D. C.—Eosinophilia is regarded as a diagnostic sign in many cases of parasitism. I should like to ask Dr. Kline whether he is of the opinion that eosinophilia is the normal reaction to a foreign protein.

DR. HAL M. DAVISON, Atlanta.—We must find the answer to the question, Can eosinophilia, either local or general, be considered typical of allergy or diagnostic of allergy? At all the allergy meetings of the last few years this question has been discussed. We always come back to the same point. We have some cases of allergy in which the patients give a typical history, typical family history and positive skin reactions and in whom good results are obtained by treatment with these materials that give positive skin reactions, yet some of these patients never show any form of eosinophilia either locally or generally. Conclusions from the experiments of Drs. Kline and Young must be based on the acceptance of the fact that eosinophilia is diagnostic of allergy.

I should like for Dr. Kline to tell us whether he considers this a proved fact or simply a hypothesis.

DR. BRET RATNER, New York.—I think the authors' statement that the appendix might be inflamed as a result of allergy gives us a note of warning. We have become rather too sanguine in diagnosing abdominal manifestations in the allergic child as due to enterospasm. As the authors suggest, inflammatory reactions may well occur in allergy. I think that this is very important.

The striking thing, it seems to me, that Dr. Kline emphasized is the fact that all of allergy seems to be acquired as the result of repeated entrances into the body of foreign proteins, which is opposed to the concept that allergy is entirely dependent upon constitutional factors.

DR. KLINE, Cleveland.—A great many of the patients presented had an apparent history of allergy, and quite a number of them were such that the history of allergy had to be found on subsequent reexamination of the patient, so that the clinical histories were frequently unreliable. I believe that the anatomic findings really are a safer guide than the best clinical history you can obtain or the changes you can determine by the most careful clinical examination.

There is no opposition to the idea of a constitutional factor in certain allergic individuals. A similar controversy concerning tumors has been decided fairly conclusively: Whereas Cohnheim and many followers believed that tumors come from anlage within the individual at birth, the numerous evidences of cell autonomy have made it clear that at any time in our lives, either before we are born or afterward, we may develop tumors. There is no opposition to the idea that susceptibility to tumor formation or to allergy may be transmitted hereditarily, but on the other hand it is a fact that hypersensitivity can be acquired. You can take a rabbit perfectly normal as far as you can determine, for instance, and make him exceedingly sensitive to horse serum.

The trouble that pathologists are having in allergic inflammation is in determining the etiologic agent. Many of the severe reactions resemble those produced by living and multiplying organisms, and whether or not found, the pathologist believes them to be present. The idea of a sterile inflammation produced by a foreign protein in a sensitized individual has a right to come to the fore now.

THE TREATMENT OF CHRONIC ATROPHIC ARTHRITIS WITH AUTOGENOUS STREPTOCOCCUS FILTRATES (ANTIVIRUS)*

Preliminary Report

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AFTER a four-year period of trial and despite the glowing reports of other workers,[1, 2] the failure of autogenous vaccine therapy to produce better than 48.4 per cent improvement[3] in the treatment of chronic atrophic arthritis prompted us to seek a more satisfactory approach to the problem of specific treatment.

Nerb[4] having successfully used antivirus in accordance with the technic of Besredka[5] for genitourinary infections applied this same method to the preparation of a streptococcus filtrate or antivirus. Using bacterial filtrates (antivirus) derived from various strains of streptococci of autogenous origin, we have been able to obtain definite improvement in 84.3 per cent of seventy patients treated. The present communication is in the nature of a preliminary report of this work. So far as we are aware, the English literature to date contains no comparable group of cases similarly treated with streptococcus filtrates (antivirus).

Only unmistakable cases of the atrophic variety were selected for treatment in view of the fact that this type is almost universally accepted by authorities as being due to infection, whereas the relation of the hypertrophic form to infection is still a moot question. Mixed types were avoided.

For many years attention has been directed to the problem of infection in the etiology of atrophic arthritis. There are numerous references in the literature to the findings of various types of streptococci of an attenuated variety in the blood, joints, and lymph glands of patients.

There has been found no constant variety of streptococcus. In 1929, Cecil, Nicholls, and Stainsby[6] claimed to have isolated the so-called "typical strain." In a series of 78 patients, these authors recovered a blood stream streptococcus in 61.5 per cent. They reported also that 83.3 per cent of the growths obtained were culturally and biologically identical, appearing to be attenuated hemolytic streptococci. In confirmation of their studies, other workers obtained positive blood cultures; notable among these were Gray and Gowen[7] (57.7 per

*From the Medical Service of The Brooklyn Hospital.

cent of cases), Wetherby and Clawson[8] (50 per cent), Klugh[9] (71.6 per cent). All of these writers, however, obtained different strains of streptococci. Moreover, other reliable workers, namely Nye and Waxelbaum,[10] Bernhart and Hench,[11] Dawson, Olmstead, and Boots,[12] were unable to duplicate in any respect the findings of Cecil and his collaborators.

With such conflicting evidence it would appear that to date there is little or no proof that chronic atrophic arthritis is caused by any one specific streptococcus. More tenable is the concept that it results from the effect of any one or several of many different strains of streptococci. In support of this is the finding in atrophic cases of high-titer agglutination to streptococcus in a group sense ([7, 13, 14, 15, 16, 17]). Studies have further borne out the fact that agglutinins when present in the serums of these patients are not strain specific. Some strains are agglutinated in higher dilution than others, but no single strain predominates.

There still exists a wide gap in knowledge of the steps which take place between the point of focal infection and the end-product of its influence, the pathologic joint. The casual finding of streptococci in or about a joint is in no way conclusive that these represent a focal point of infection in that joint. Such organisms are probably blood borne but conceivably deposited in or about the joint by accident. Far more in keeping with the local pathologic joint findings which are not the picture of the inflammatory reaction of local infection, is the theory that the process is an allergic one, the allergen being a bacterial product originating from some remote point and influencing the joint as a sensitized tissue.

Freiberg[18] demonstrated that an atrophic arthritis can be produced in animals on an allergic basis when he noted in a rabbit following the repeated injection of bacterial filtrate the development of a joint lesion pathologically resembling the atrophic joint in human beings. This monarticular arthritis did not develop until two or more injections of bacterial filtrate had been given; with successive injections the focal reaction in the joint became more and more pronounced. Coincidentally, there developed in the rabbit not only specific blood agglutinins as indicated by high-titer serum agglutination to the original bacterium used in preparing the filtrate, but also a skin hypersensitivity to the filtrate.

Following these studies, Freiberg and Dorst[19] reported their preliminary clinical results in the treatment of thirteen cases of atrophic arthritis by the desensitization method. Cures were effected in seven, marked improvement in three, some improvement in one, and no improvement in two.

Freiberg and Dorst used filtrates obtained by the growth of organisms cultured from various foci of infection. The patient was skin

tested individually to these filtrates. Those organisms the filtrates of which gave positive intradermal skin tests were used for therapy, "antigens" being prepared from the organisms themselves. Very small amounts of antigens in high dilution were injected subcutaneously every one or two days. Cases selected for treatment were only those which had not progressed to severe bony deformity and which reacted positively to intradermal skin test. Although Freiberg's series was small, the results were sufficiently striking to foster the concept that in some cases of atrophic arthritis at least there is a relationship between antigenic substances recovered from distant foci and the diseased joint as a shock tissue.

For four years patients of the arthritis clinic of the Brooklyn Hospital had been treated with what were deemed to be autogenous vaccines. Complement fixation tests produced no assurance that the strains obtained were etiologic. Intradermal skin tests to the whole organisms in suspension were negative, some forty-four patients with atrophic arthritis having been tested to their own growths of streptococci. From this it would seem that vaccine prepared from points of focal infection may bear absolutely no relationship to the disease. Certainly, it is possible to obtain as many varieties of streptococcus from about the tonsils and teeth of healthy individuals as from those of arthritic individuals. Which, if any, of the organisms thus found is responsible becomes a very real problem.

Short, Dienes, and Bauer[20] who recently carried out skin tests with vaccines prepared from strains recovered from foci of arthritic patients found that these strains did not yield positive skin tests—confirming our own experience. The selection of effective strains by this method does not seem feasible.

After the virtual failure of autogenous vaccine therapy in our hands, the next step carried out was the preparation of a stock bacterial filtrate (antivirus) derived from a group of strains of streptococci isolated from foci of infection in arthritic patients. Intradermal injection of this conglomerate filtrate in 0.1 c.c. doses in atrophic arthritis patients produced within twenty-four hours positive skin reactions. Each patient had a broth control injection in the other forearm. Fourteen apparently normal physicians were similarly skin tested as a control—with negative results in all save one. Twelve cases of pure hypertrophic arthritis skin tested with the same technic yielded negative results. From these findings it was suggested that patients with atrophic arthritis show skin hypersensitivity in a group sense to bacterial products of streptococcus, a reaction such as is generally not found in normal controls.

Subsequently fifteen patients with atrophic arthritis were treated with this stock streptococcus filtrate with the following results: Three

were made symptom free; three were greatly improved; four, slightly improved; five were unimproved or made worse.

It was thought that possibly more specificity might be established if autogenous filtrates rather than stock were used.

The following method is used at present: Every case has all recognizable foci of infection cultured, viz., nose, teeth, gums, tonsils, pharynx, stool, and in some cases prostate and in others the cervix. These are planted in ordinary broth. The broth is made from beef heart muscle and contains peptone and sugar. The cultures are allowed to incubate from twenty-four to forty-eight hours and are then transferred to blood agar plates. Pure strains of streptococci are isolated, identified and individually reinoculated into beef heart broth and again grown for twenty-four hours. They are then transferred to savita (yeast extract) where they adapt themselves to a slow growth. This step is taken because savita does not contain peptone. (Originally when we started this work, we found that some patients already were or soon became sensitive to the broth controls.)

The bacteria grow on savita media for twelve to fourteen days, the pH being maintained between 7.2 and 7.5. The cultures are then centrifuged and put through a Berkefeldt filter. The resulting filtrates thus obtained are used for the skin testing and subsequent treatment. A separate filtrate is made for each type of streptococcus identified. These filtrates contain so far as we know exotoxins elaborated in the growth of the streptococcus and to a certain extent some of the endotoxins and nucleoproteins of the organism. What exact constituents of the filtrate are responsible for the positive skin reactions we are at present unprepared to say. We are certain by adequate control that the positive skin tests are related to bacterial products. The amount of total protein in our filtrates was calculated to be 23 mg. per 100 c.c., or approximately the protein content of normal spinal fluid.

Having obtained these individual filtrates, the patient is skin tested to each, using 0.1 c.c. of savita as control. Only those filtrates producing a positive reaction at the end of twenty-four hours are selected for treatment. The positive reaction consists of a small central area of induration and of a large surrounding area of erythema. In addition to this there may occur an exacerabation of pain in the joints. Depending on the intensity of these reactions the positive filtrates are combined and diluted. The usual initial injection is 0.1 c.c. of a 1:100,000 dilution. This is given subcutaneously. In a few instances focal or systemic reactions to this injection have necessitated beginning with a 1:1,000,000,000 dilution. Following the initial dose, treatments are given twice weekly, and the dosage is increased by 0.1 c.c. until 1 c.c. of the dilution has been given. In similar manner the

patient is carried through dilutions of 1:10,000, 1:1,000, 1:100, 1:10, until the full strength filtrate is reached. The treatments are then given once a week.

Focal reactions have been noted during the course of treatment in 29 of our cases. These were characterized by an exacerbation of pain in the affected joints within twenty-four hours after the injection. When this occurs, the subsequent dose is not increased but maintained at the same level. We look on this focal phenomenon as a favorable omen, because it suggests that in the filtrate are substances to which the patient is not only skin sensitive but joint sensitive.

Seventy cases of atrophic arthritis have been treated in the above mentioned manner. The results are indicated in Table I.

TABLE I

Recovered	9 – 12.9%		
Greatly improved	25 – 35.7%	84.3%	
Slightly improved	25 – 35.7%		
No improvement	11 –		15.7%

Nine of these have recovered or are asymptomatic.

It should be emphasized that the disease is chronic and that periods of remission and exacerbation are characteristic of its life history. How long these patients will remain "cured" one cannot predict; adequate follow-up alone will establish the criterion of cure.

The present percentage of improvements is the largest which has to date been obtained in this clinic with any type of so-called "specific therapy."

TABLE II

RESULTS OBTAINED IN RELATION TO THE DURATION OF THE DISEASE

	LESS THAN 1 YR.	1-2 YR.	3-5 YR.	6-10 YR.	OVER 10 YR.
Recovered	5	3	0	1	0
Greatly improved	5	10	3	2	5
Slightly improved	8	7	5	2	3
No improvement	0	0	3	4	4

A comparison of the number of cases improved with the duration of symptoms before treatment (Table II) established the fact that there have been no failures in cases in whom the duration of disease was less than three years. This suggests that if treatment is started early, good results may be expected.

CONCLUSIONS

1. There is no definite evidence that atrophic arthritis is caused by any one specific type of streptococcus. The evidence seems to indicate that the disease results from the effect of different types of streptococcus localized in varied foci of infection.

2. It is likely that the reaction in the joints to the products of these organisms is an allergic one. In support of this it has been demonstrated that cases of atrophic arthritis will react with a positive skin test to filtrates prepared from the strains of streptococci isolated from their own foci of infection. It is to be emphasized that bacterial filtrates and not vaccines are to be prepared for skin test and therapy.

3. Eighty-four and three-tenths per cent of the patients with atrophic arthritis treated by desensitization to autogenous streptococcus bacterial filtrates (antivirus) to which they showed positive skin test improved; whereas only 48.4 per cent of patients treated with autogenous streptococcus vaccine showed improvement.

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STUDIES ON THE RELATION OF THE ADRENAL GLANDS TO ALLERGIC PHENOMENA*

3. On the Specific Therapeutic Effects of Cortical Adrenal Extract in Asthma

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THE remarkable therapeutic effects of epinephrine in the asthmatic attack focus attention on the medullary portion of the adrenal gland. The loss of appetite and asthenia complained of by so many chronic asthmatic persons, the hypochlorhydria and low blood pressure simulate the symptoms of adrenal cortical insufficiency and suggest the use of cortical extracts in this disease.

Early in 1930 we administered a potent cortical extract to a boy twelve years of age, an asthenic chronic asthmatic, whose attacks were due to contact with feathers, house dust, and orris root. Environmental control of contact with these substances over a period of several months failed to stop the symptoms and hyposensitization with these substances was considered. Before carrying this out, however, he was placed on a cortical adrenal extract (Interrenalin) by mouth in dosage adequate to relieve the symptoms of Addison's disease in adult patients, and all other symptomatic treatment was discontinued. During a five weeks' trial of this substance there were severe asthmatic attacks and no significant change in the general condition. The extract was at this time discontinued, and hyposensitization was carried out.

Since a demonstrably potent extract had failed to affect the asthma in this case, and since the extract was expensive and difficult to prepare, it was not considered worth while to administer it to any other asthmatic patients.

In March, 1933, Fineman[1] published his observations on the use of a commercial cortical adrenal extract (Eschatin) in four asthmatic patients. He reported that one showed marked improvement, one moderate, one slight and one no improvement. He felt that, "The results obtained with suprarenal cortical extract in the four cases studied and reported warrant its further trial in a larger series of cases, employing larger dosage and utilizing, if necessary, the intravenous route."

Because of this report we decided to make further observations. Accordingly we administered Interrenalin for a period of six weeks

*From the Asthma and Hay Fever Clinic of the Asthma, Hay Fever and Allergy Foundation.

to three adult chronic asthmatic persons. Two of these patients were very thin and asthenic, unable to do any work, scarcely able to move about, using large amounts of epinephrine daily in repeated small doses for only moderate relief. The third patient resembled the two others in all respects except that she was obese. As in the case of the patient previously described, the extract was administered by mouth in amounts more than sufficient to ameliorate the symptoms in adults with Addison's disease. In no case was there the slightest change in the severity of the asthma as determined clinically or by the amounts of epinephrine needed to control the symptoms.

Studies of the effect of any therapeutic agent in a chronic disease, especially one like asthma in which there are likely to be periods of remission, require most careful control. Many reports of beneficial results following some particular treatment can be explained by the lack of knowledge of the variations in the diseases in the patients being studied. For this reason these patients were selected from those whom we had failed to relieve by other methods of therapy, and whom we had observed for periods from nine months to two years, and in whom we had accurate knowledge of the periodicity of their symptoms.

If relief had been obtained, we could, therefore, have been reasonably certain that it was due to the extract which was administered.

The extract used in this study was furnished us by Dr. J. M. Rogoff. It was part of a supply prepared by him for use in animal experimentation and in the treatment of patients with Addison's disease. Since there are no generally acceptable standards for chemical or physiologic standardization of adrenal extracts for use in man, it seems fair to accept an extract as potent which will materially lengthen the life expectancy of patients with Addison's disease or has proved to prolong life of bilaterally adrenalectomized dogs. In most of the published reports on other extracts (Cortin-Eschatin) they were administered in huge doses parenterally to patients in the so-called acute crises of Addison's disease together with physiologic salt solution, glucose solution or both, and many recovered from the crises. Since Addison's disease runs a course with crises alternating at times with remissions, and since recovery from crises often follows the administration of large amounts of fluid without any cortical extract therapy, the beneficial effects of such therapy must be judged by the prolongation of life in a large series of cases in which the cortical extract is administered without complication by saline or other injections. Such a series has been published by Rogoff[2] who studied patients treated over a long period of time with Interrenalin by mouth with definite evidence of a marked increase in expectancy in a treated group as compared with an untreated group observed simultaneously. The untreated controls died at average times almost identical with those pub-

lished by Addison, Kovacs, and others. There can be no doubt therefore of the potency of the extract used for these experiments.

CONCLUSIONS

Although the series of cases is very small, the observations in these four cases indicate clearly that cortical adrenal extract has no significant specific effect on asthma in patients with continuous attacks in whom the vagaries of the disease are known.

We were concerned only with the asthma in these cases and did not study the effects of the extract on the weakness and asthenia.

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HISTAMINE AND TYPHOID PROTEIN COMPARED IN THE CONTROL OF ASTHMA AND HAY FEVER*

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NEW ORLEANS, LA.

IN A COMPLETE analysis of results obtained in our allergy service at Charity Hospital, we found patients who carried characteristic marks of allergy inheritance and who had specific skin reactions, but in whom appropriate extracts in large doses effected but temporary relief. Fifty per cent of these patients were then given histamine, and the other 50 per cent were given partly digested typhoid vaccine, and the results were sufficiently encouraging to justify this report. Histamine has been used for two and a half years and typhoid antigen for eighteen months.

In the vast literature on the treatment of asthma with histamine and various nonspecific proteins, the more firmly established results may be found.[26, 38, 22, 13, 8] The nonspecific curative effect of acute inflammations on psoriasis and lupus has its counterpart in asthma, which is frequently relieved by acute attacks of various infections, such as pneumonia, etc. The prompt termination of asthma after intravenous injection of standard triple typhoid vaccine has been observed by various workers. This caused us to use typhoid bacilli for the preparation of the antigen used in the present series of cases.

Many patients with hay fever have heretofore shown a variable period of freedom from symptoms following minor surgical operations (Charity Hospital Reports, Allergy Clinic). In these cases, reabsorption of serum or extravasated blood no doubt acts as the nonspecific agent. Although on a smaller scale, this is apparently a process similar to that causing traumatic shock following severely lacerated and hemorrhagic wounds.

The typhoid protein used in this series was prepared by digesting with an aqueous solution of KOH the killed, dried typhoid bacilli. Then, by the use of alcohol, a fraction containing, principally, the cellulose-like material was precipitated and removed by filtration. The remaining protein fractions in the filtrate were standardized by nitrogen content and the pH was adjusted to 6.8. This product was ready for use after being put up in a sterile condition in rubber-capped vials.

Care has been exercised in the preparation of the typhoid protein to ascertain that the solution was always clear and free from precipitates, and to see that it was at the desired hydrogen ion balance. We did not employ the typhoid protein in aged patients nor in those whose re-

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sistance seemed to be extremely low. Shock and allergic manifestations were controlled by the prompt use of adrenalin.

Warning is given that the intravenous injection of typhoid bacilli is not without danger, for the floating clumps of bacilli might well produce serious or fatal embolism. The severe protein shock reactions which follow the use of typhoid vaccine and other nonspecific proteins are likewise not without grave danger.

However, we find that this protein is a safe and effective agent for the treatment of asthma. It has been used in the very refractory asthmatic and hay fever cases with encouraging results. Where all other remedies have been tried, found ineffective, and discarded, we have used the typhoid protein successfully. It has not been utilized in cases where specific pollen reactions indicate the use of specific pollen antigens. In such cases there is no reason to believe that it would be superior in any way to the specific pollen.

We have adopted a plan of using small doses repeated at short intervals. Eleven years ago Bel[5] pointed out the advantage of this plan.

Histamine, the antigen used for the other half in this series, is a drug of potency excelling that of other previously used nonspecifics in allergic conditions. We have found histamine especially useful in cases of allergic skin disturbances, in hay fever as well as in asthma.

Bronchioles are strongly constricted (anaphylactic-like action) by histamine,[2, 3] although it is alleged that on the normal musculature this effect may not be seen.[27, 44, 45] It is a powerful vasodilator[21, 34] and induces marked permeability of the blood vessels (inflammatory phenomenon) with fall of blood pressure (essential phenomenon of traumatic shock).[28] One drop of histamine applied to the slightly abraided skin produces the typical urticarial wheal. Some regard it as a circulatory regulator.[21, 23, 34, 42, 31] Calcium, sodium, etc., are synergists,[17] while acids inhibit its action. Histamine is formed as a result of the action of putrefactive bacteria on protein, but it has been found also in normal tissues of the human body, especially in intestinal mucosa and particularly in pyloric mucosa.[1, 4, 37]

After histamine is injected, the blood stream loses some of its plasma to the surrounding tissues, excluding scar tissue.[7, 35] A previous local injection somewhat modifies this response.[36] One-tenth of a cubic centimeter of a 1:1,000 solution of histamine suffices for a circulatory test[10] except when spasm of the vessels occurs. Increased calcium content in the tissues has been found to influence the test.[31, 44] Disregard of this feature may explain the apparent lack of harmony of various reports on the subject.[31, 34] In inflamed areas its effect is heightened,[23] and bleeding at the site is more likely to occur.[34]

Like adrenalin, trembling follows excessive administration of histamine.[34] Cortin[17] and adrenalin[6, 11, 25] have been found to be histamine's direct antagonists. Formaldehyde neutralizes it.[16]

The use of histamine is by no means devoid of danger, as ulcer of the stomach has been produced by it,[19] and fatal syncope might well occur with its use. The lethal dose is 1.2 mg. per kg. of body weight.[32] The smallest effective dose is 1:200,000,000 dilution of blood.[6, 14, 41, 42]

Table I shows the results obtained in 110 cases of asthma and hay fever, though we treated 84 patients with various forms of allergy with typhoid protein and 5 patients with histamine, 135 in all.

TABLE I

	ASTHMA TREATED WITH TYPHOID PROTEIN 80 CASES	ASTHMA TREATED WITH HISTAMINE	HAY FEVER TREATED WITH HISTAMINE
		30 CASES IN ALL	
Spring, improved	15%	30%	14%
Fall, improved	40%	10%	42%
Perennial, improved	25%	50%	42%
Unimproved	10%	10%	2%
Aggravated	10%		

After applying histamine two and a half years and typhoid protein for eighteen months, a questionnaire was sent out. The replies (in 110 cases), gathered just at the termination of a severe asthma season, may well be considered dependable.

In looking for interesting examples to illustrate the effect of typhoid protein, we examined in detail 84 particularly refractory cases in which vaccine, diet, specific pollen and hygienic measures had failed. Six hundred and fifty treatments were administered, making an average of 8 doses for each patient, although some received 30 doses. One patient was ill less than a year, while some had been suffering for forty-seven years. Sixteen were given one dose; 49 discontinued treatment before the tenth dose.

The 51 cases of patients with gastrointestinal allergy, hay fever and asthma treated with histamine were compiled. They had symptoms from two months to twenty-five years, and received a total of 523 injections. The average dose was 0.5 c.c. of a 1:1,000 solution once or twice weekly. The average number of injections (excluding patients who received less than five) was 10. One received 59.

Some illustrative case reports follow:

CASE 1.—N. S., a white male, aged eleven years, had allergic asthma. Following an attack of pneumonia, his first attack of allergic asthma came on suddenly with dyspnea and weakness at the age of two years. Since then, independent of the season, each time he was chilled when perspiring freely, he had an acute spell. In 1930 his tonsils and adenoids were removed without relief. After a paroxysm, we found the usual

physical and x-ray signs in the chest. He gave a positive skin reaction to ragweed. Anemia was marked. No other pathologic condition was found. He failed to improve with the specific pollen injections. He was given 25 doses of typhoid antigen with a maximum of 0.5 c.c. subcutaneously, twice weekly. He has been entirely free for one year, and reports at intervals for a recheck. His anemia has cleared and his weight and activity are normal.

This experience is a repetition of the result obtained in children. Vaccines and other nonspecifics act well. The tonsillectomy undoubtedly was necessary but was not effective alone.

CASE 2.—M. J., a white female, forty-five years of age, weight 165 pounds, was a typical case of allergic (grass) bronchial asthma. Her first attack occurred in 1923. She was first seen in 1931 during a distressing paroxysm which came on suddenly at night and was followed by a moist bronchitis. There was no loss of weight, and between attacks the patient appeared well. Most of her time was spent at her home, but nothing there or in her habits gave a clue to the cause of her illness. We presumed that the climacteric had intensified her asthma. Extracts, vaccines, and palliatives produced temporary relief. After 25 injections of typhoid protein she definitely improved. After the fifteenth dose her attacks became less marked. She last reported four months ago; her attacks had ceased and she has been clinically well for eight months.

CASE 3.—Mrs. J. H., white female, aged forty-five years, was a typical case of severe asthma. We have treated her for nineteen of the twenty-four years she has been suffering. With pollen extract and vaccine the attacks were restricted to the winter months, but were still severe, and two years ago she had her worst attack. We then substituted histamine and the patient received 22 doses. She improved so much that since that time she has had only two light attacks. We see her at regular intervals.

CASE 4.—F. R., white female, had allergic hay fever and asthma. Although of allergic stock, and giving positive skin reactions to pollens and wool, her symptoms became marked only with the advent of the menopause. At forty-five years, when she presented herself for treatment, she had had hay fever for one and a half years and asthma for nine months. On physical examination we detected no organic defect except in the lungs, which showed a marked bronchial asthma. From January, 1932, to March, 1934, she received 30 injections of histamine, the highest 0.6 c.c. of 1:1,000 dilution. Her last attack was in November, 1933, having had two others, one in July and the other in October, 1932. She has gained 15 pounds and is apparently well. On one occasion, shortly after receiving 0.4 c.c. histamine, she suddenly became cyanotic and

suffered acute dyspnea; adrenalin 0.35 c.c. relieved her at once. Previous to the histamine injections, extracts (grass and ragweed) had relieved her for only short intervals.

Histamine improved 95 per cent of the patients, while typhoid protein relieved 83 per cent. The best results in perennial asthma were obtained by the use of histamine, while in cases of fall asthma, typhoid protein was most effective. Preference, however, is always given to the proper pollen injections when specific reactions indicate their use.

SUMMARY

Although our experience with hyposensitization, extending over a period of eighteen years, has taught us conservatism, we find that we can safely conclude that:

1. Typhoid split-protein and histamine are both useful in the treatment of allergic cases, be they skin, respiratory, bacterial, or digestive.

2. Typhoid split-protein is much safer than histamine, although not quite so rapid nor so powerful in its effect.

3. Small intradermal doses of either will work better and more safely than large doses.

4. Histamine injections may precipitate acute asthma; this, however, can be easily controlled with adrenalin.

5. Histamine in the aged or arteriosclerotic, if used at all, should be employed carefully in minute doses.

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"HONEY DEW" FROM THE LIVE OAK A CAUSE OF HAY FEVER AND ASTHMA

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IN THE regions in which there is such an abundance of evergreen foliage as in this section of the country, there is evidence of a new source of pollution of the air which has thus far escaped the attention of the medical profession as a cause of hay fever and asthma. We refer to a large family of foliage pests of the order of *Hemiptera* which are prevalent in the live oak trees. These insects excrete "honey dew" into the atmosphere.

For the past fifteen years we have noted and remarked upon the failure to desensitize successfully the many patients who are obviously oak sensitive. In contrast to this failure we have successfully treated patients sensitive to grass and ragweed pollens. Further, it was especially noted that many sufferers with hay fever and asthma developed their attacks well in advance of the oak pollen season or in advance of any pollen in the atmosphere. There was no cause for this strange situation evident until it was noted that numerous fine droplets appeared on the glass windshields of automobiles coincidentally with the periods of suffering among many of the hay fever and asthma subjects. We have observed this for the past three years.

With these facts in mind, we shall present a study of the subject. We realize, however, that such an early report as this, without a number of cases showing the successful application of the principles which we present, offers an opportunity for criticism, and to the charge of overconfidence. We believe, however, that the clinician is the field explorer and the field worker. We deem it his province especially to report the clinical aspects and the general points and indications for later and more thorough survey, and for rectification by technical workers in this department of medicine. We are convinced, for instance, that oak sap antigen requires the extraction of tannic acid. This is work for the technician, since our training and duties as clinicians do not permit us fairly to enter the field of pharmaceutics.

Honey dew is described as a sticky sweetish fluid ejected by the foliage pests. It is stated by students of plant pests that ants are found ascending a tree infested by these insects on one side of the tree, and on the other side ants are found descending filled to the utmost with the honey dew which they have secured. In this way is determined the need of spraying for these blights. The ants obtain the honey dew by stroking with their antennae two protuberances at the posterior end of the insect. This tremendous industry on the part of the ants there-

fore would reasonably offer the source of an abundant escape of the practically pure live oak sap, which would allow droplets in the air under the trees, even though the digestive function of the insects should be capable of destroying the protein content without this phenomenon induced by the ants. A great abundance of these minute droplets may be observed frequently upon automobile windshields, and may be gathered upon microscopic slides placed under the trees. These sticky saplike droplets present under the microscope small globules of resinlike substances. Likewise the sap gathered from the thick juicy bark at the trunk of the live oak presents the same globules and appearance.

The source of the honey dew is from the green foliage. The insect has a sharp proboscis which is inserted into the stomas of the leaves, where the sap supply for the growth of the leaf is found. The sap flows upward in the tree in the outer layer of the bark and that which is not utilized in the foliage and products of the twigs is returned in the cambium layer to nourish the roots.

Wherever the evergreen hardwood trees abound or wherever foliage is abundant an opportunity will be offered for showers of the honey dew; in this section of the country where the live oak is in preponderance, there is a frequent recurrence of the balmy weather which enables these insects to thrive, particularly when the sap is flowing freely. A shower of these droplets was noted on Christmas morning in 1933 after an outbreak of hay fever had been noted.

As stated before, for fifteen years we have noted and remarked upon the failure to secure satisfactory relief in the cases regarded as oak pollenosis. In addition we have noted, as before mentioned, that the onset of attacks came sharply and well in advance of the appearance of any budding or formation of catkins and pollen. The honey dew pollution of the air in Mobile, where the live oaks form a canopy-like foliage over the streets, gives abundant indications for guarding the patients sensitized to oak by early and perennial treatment.

For some years we have noted that the oak pollen antigen failed to give a definite and characteristic reaction as compared with the cases of ragweed pollen sensitization; yet there was no doubt that the sufferers were oak sensitive. This seemed to explain in a way why there was failure in treatment. However, since the droplet pollution of air as shown on the automobile windshields had appeared so early, it was thought that desensitization had not been started sufficiently early. The attempt to correct this by earlier preseasonal treatment and then the continuation of the monthly injections, recommended under the perennial system, again failed to secure the hoped-for results. We then attempted to secure a better antigen than the one provided from the oak pollen. We made a crude crush preparation of leaves in Coca's buffered salt solution, and another crush preparation from the green bark scraped from the last twig growth. Both of these gave a more characteristic

positive reaction than the pollen antigen. Later, however, in trying to make enough extract from leaves and from the bark of the twigs we were disappointed in finding that it did not produce the marked reaction which was produced by the droplets obtained from wiping off the windshield. Finally, and quite recently, we obtained the oak sap in generous amounts from the juicy bark between the rough knuckles at the trunk of the tree. The skin reaction was decidedly the most pronounced and characteristic to this, thus giving substantial proof that a better antigen was to be had in it.

We have had opportunity to apply the improved antigen in only one case. The first dose of the oak sap antigen caused a large intense reaction. Thus far five doses have been injected. The local reaction has lasted longer than any reaction of the kind, even that from ragweed, to which the patient is very sensitive. We therefore have not repeated the doses oftener than at about four- or five-day intervals, and we have had to reduce the initial dose by half. The suffering both with distressing hay fever (which required several handkerchiefs each morning) and with asthmatic breathing (which demanded relief each night by one or two doses of adrenalin) was at once relieved, and the relief has continued over two weeks. We had been giving the pollen antigen up to the time of starting the sap antigen, but no marked reaction appeared locally and no relief was brought about. Nevertheless, we thought it best to continue until we obtained a better antigen because of the more dangerous season ahead from both the honey dew and the pollen.

In closing we wish to acknowledge the assistance of Mr. H. P. Loding, an expert botanist and a student of plant pests. We had had the impression that the droplets on the windshield and the droplets gathered from under the trees were directly escaping oak sap. Especially did this seem to be evident when the same resin globules were identified so readily in each. Mr. Loding told us that this could not be the case, but stated that we were recovering honey dew, as we have described here. He also kindly studied under the microscope the droplets which were gathered from the windshield of our car and compared these droplets with the oak sap secured directly. He found the appearance very similar in that each showed the same resin globules. On the other hand, the honey dew which he obtained from the aphis on snapdragon leaves showed a different character than the honey dew from the oak, with no resin globules.

We hope that this report may draw the attention of other workers and may result in better success with the oak sensitization cases of hay fever and asthma.

We wish to acknowledge the assistance of Mr. J. A. Casteel, pathologist, in making the extracts referred to above.

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CUTANEOUS ALLERGY FOLLOWING THE INGESTION OF AMIDOPYRINE

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SINCE R. R. Kracke[1] first reported severe and repeated attacks of agranulocytosis in an individual using large amounts of coal tar derivatives, and especially since the later and more extensive reports of Madison and Squier,[2] the belief that amidopyrine may in sensitive individuals produce agranulocytosis has been gaining ground. The behavior of these patients makes it highly probable that the process is an allergic one, but, if so, it is surprising that so few cases of urticaria and angioneurotic edema following the use of amidopyrine have been observed.

A search of the literature is rewarded by only two such cases. In 1930 Crohn[3] recounted an attack of urticaria and angioneurotic edema in himself following the ingestion of two allonal tablets. He added the following comment: "Since on former occasions I have borne pure veronal as well as pyramidon without symptoms, these two ingredients may be excluded" as responsible for the symptoms. That this conclusion is not justified, my own case will illustrate.

The second case is that of Unger[4] whose patient, a hay fever sufferer, broke out with urticaria and angioneurotic edema the morning after having taken three allonal tablets. Twelve days later the same thing happened a day after taking six grains of pyramidon. Skin tests with amidopyrine, as well as with a number of other drugs, were negative.

The following case differs in some respects from both of the above.

A. T., male, aged sixty-three years, is sensitive to tree, grass, and ragweed pollen and has for many years been receiving grass and ragweed pollen injections. A maternal aunt died of agranulocytosis following the taking of allonal tablets.

Since adolescence he has suffered from frequently recurring headache of undetermined origin. During the past eight years he must have averaged 100 five-grain tablets of amidopyrine annually. About a year ago he decided to discontinue the use of amidopyrine, though the drug had never produced any unpleasant effects.

In January, 1934, he was awakened by headache and, still half asleep, took a five-grain amidopyrine tablet. Within a few minutes a mild and brief urticaria set in, involving chiefly the upper extremities. In March the same experience was repeated, the urticaria being more severe but still brief.

On June 26, about 3 P.M., being annoyed by a headache while at work and no aspirin being available, he took a five grain tablet of pyramidon (Metz). Within a very few minutes a fairly severe anaphylactic seizure ensued: a fine coalescing urticaria involving only the upper extremities, an angioneurotic swelling of both lips, especially the upper, a conjunctival injection with blurring of vision and a mild degree of bronchial asthma. He was immediately given a little less than 1 c.c. adrenalin hypodermically, whereupon the symptoms subsided almost as quickly as they had come, leaving behind only a little aching in the knee joints. A white count taken while the symptoms were subsiding resulted as follows:

White cells 8,300

Ba	Eo	My	Ju	St	Se	Ly	Mo
0	4	0	0	4	42	46	4

Twenty-four hours later, subjectively well, the count was:

White cells 5,100

Ba	Eo	My	Ju	St	Se	Ly	Mo
1	2	0	0	4	49	43	1

After another twenty-four hours, subjectively well, the count was:

White cells 5,300

Ba	Eo	My	Ju	St	Se	Ly	Mo
0	6	0	0	0	43	48	3

Two days later the white count had risen to 6,100 and has remained normal ever since. A patch test, a scratch test and an intradermal injection of a saturated watery solution of amidopyrine gave completely negative reactions. Passive transfer was not attempted.

Nearly all observers have failed to obtain positive skin tests in cases of amidopyrine sensitization. Squier and Madison[5] alone have succeeded in obtaining positive patch tests in two cases of agranulocytosis following amidopyrine administration. In these cases a temporary granulopenia accompanied the positive patch tests. Attempts at passive transfer have failed in the hands of all observers.

SUMMARY

A case is reported of an allergic individual who, after taking amidopyrine for years with impunity, took five grains after having abstained from the drug for some months. Almost immediately urticaria, angioneurotic edema, bronchial asthma, conjunctival swelling, and possibly retinal edema set in but yielded promptly to adrenalin. A mild granulopenia, lasting several days, followed. All skin tests were negative to amidopyrine.

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PSORIASIS AS A POSSIBLE ALLERGIC MANIFESTATION

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PSORIASIS is a chronic skin disease with an obscure etiology, but there is at least some evidence in support of the theory that it is an allergic manifestation. Oriel and Barber[1] have obtained a proteose-like substance from the urine of patients suffering from various allergic and febrile conditions to which these patients were found to be hypersensitive. Desensitization with varying dilutions of this substance is said to be of therapeutic value, and psoriasis is one of the conditions reported by them to have been successfully treated in this manner. Mienicki and Ryll-Nardzewski[2] have found that the intracutaneous injection of Dmelcos vaccine, and in some cases *Staphylococcus aureus* vaccine, produces an anaphylactoid shock or hemoclastic crisis in psoriatic patients, whereas a leucocytosis occurred in the normal controls. Local cutaneous reactions in the form of psoriatic papules occurred at the sites of these injections. Zeidler[3] has noted that in patients suffering from both psoriasis and pollen hay fever, both conditions were frequently relieved by pollen therapy alone. He found that as the skin sensitivity decreased following treatment, there was a comparable decrease in the activity of the psoriatic lesions. Various forms of nonspecific protein therapy have been used with more or less success in the symptomatic treatment of psoriasis.

Several attempts have been made to obtain a specific substance from the scales of psoriatic lesions with which desensitization or antibody stimulation could be accomplished. In 1930 Campbell and Frost[4] treated fifteen cases of psoriasis by the intramuscular injection of a 1 per cent suspension of autogenous scales in alcohol. The dosage varied from 1 c.c. to 4 c.c. given at three or four day intervals. All patients responded in varying degrees to this treatment. In 1931 Stillions and Lawless[5] reported eight cases treated by the technic of Campbell and Frost and concluded that no benefit was to be derived from such treatment. In 1932 Elkin[6] reported the use of various scale preparations in the treatment of psoriasis. Three types of scale suspensions were used. The first consisted of a 1 per cent suspension of unsterilized psoriasis scales in normal saline. Subcutaneous injection of this mixture produced a severe local reaction, with some febrile response but no therapeutic effect. The second consisted of a 1 per cent suspension of autoclaved scales in sterile saline. Subcutaneous injection of this preparation produced no local or general reaction and no therapeutic effect. The third consisted of a 1 per cent suspen-

sion of the scales in alcohol, which was administered intramuscularly; one of nine cases recovered completely within one month, and the other eight showed varying degrees of improvement. Wrong[7] reported ten cases treated by the injection of scales. In one case the eruption entirely cleared, in five cases improvement occurred, and in the remaining four cases there was no improvement.

In considering the above mentioned work the intramuscular injection of alcohol alone would probably precipitate tissue proteins in the vicinity of the injection, hence produce a foreign protein shock which might, in a nonspecific manner, account for the improvement in this group of cases. The subcutaneous injection of unsterilized scales might be dangerous even if satisfactory therapeutic results were obtained, while the subjection of the scales to the autoclave would probably alter or destroy any antigenic quality of the orginial scales.

In order to investigate further the question of an antigenic factor in the scales of psoriasis lesions the following experiments were performed. Scales were collected with a minimum admixture of blood, serum or other foreign substances. The lipoids were removed with two washings in ether, and the dried scales were pulverized in a mortar. Three types of extracting fluid were used: normal saline, buffered saline, and alkaline saline. Apparently the latter yielded the most satisfactory extract. Five grams of the pulverized scales were placed in 100 c.c. of the extracting fluid and kept in the ice box for five days. The extract was then passed through a Seitz filter and proved by cultural methods to be sterile. Chemical analysis revealed a nitrogen content of 0.395 mg. per c.c. This product was slightly irritating to the normal skin; therefore dilutions 1:4 and 1:8 with respective N contents of 0.1 and 0.05 mg. per c.c. were made. Intradermal injection of these two dilutions gave negative reactions in ten normal skins.

Six cases of psoriasis were available and *in each case* positive reactions occurred at the sites of the intradermal injection of 0.02 c.c. of the weaker dilution (N 0.05 mg. per c.c.). The presence of atopic reagins in the blood of these individuals was not demonstrable by passive transfer, nor were precipitins demonstrable in vitro.

CASE REPORTS

CASE 1. White male, aged thirty-seven years. General health good, no family or personal history of allergy. No family history of psoriasis. Typical psoriatic lesions moderately distributed over back, legs and arms for twenty-three years without a complete remission. The initial dose was 0.1 c.c. of the N 0.05 dilution, and at four to seven day intervals the dose was increased until the fifteenth injection consisted of 1.0 c.c. of the N 0.4 dilution. At this time the skin was smooth with the exception of two small areas about one centimeter in diameter

on the anterior aspect of the right leg. No scales occurred elsewhere on the body, but pigmentation revealed the sites of the previous lesions. The intradermal skin test at this time is negative.

CASE 2. White male, aged sixty years. General health good. No family or personal history of allergy, although a son suffers with ragweed hay fever. No family history of psoriasis. Psoriasis is limited to a small area on the extensor surface of the right forearm. Duration thirty to thirty-five years. The initial injection consisted of 0.1 c.c. of the N 0.05 dilution and was increased at two or three day intervals until the twenty-fifth dose, which consisted of 2.0 c.c. of the N 0.4 dilution. At this time the lesion was red, there were no bleeding points, but some scaling persisted. In this case definite improvement occurred, but the results are not considered satisfactory. The intradermal skin test is negative.

CASE 3. White male, aged twenty-seven years. General health good. No family or personal history of allergy. His father and maternal grandmother suffered with psoriasis. Psoriasis was extensive, covering most of the body; duration one year. Before the injections were started the patient had received a series of x-ray treatments with great improvement, but areas not exposed to the x-ray presented typical active lesions of psoriasis. The initial injection consisted of 0.2 c.c. of the N 0.05 dilution, and the dose was increased at three or four day intervals until the nineteenth injection, which consisted of 2.0 c.c. of the N 0.4 dilution. At this time the skin was smooth, and there was no evidence of the previous lesions except for pigmented areas at the sites. The intradermal skin test is negative.

CASE 4. White male, aged thirty-eight years. General health good but has had pulmonary tuberculosis, which apparently has been arrested for several years. He suffered with bronchial asthma prior to three years ago. A grandmother suffered with asthma and a sister is subject to hay fever. There is no family history of psoriasis. Psoriasis is extensive, involving body, extremities, face and scalp; duration sixteen years. The initial injection consisted of 0.2 c.c. of N 0.05 dilution and was increased at three or four day intervals until the fifteenth injection, which consisted of 1.0 c.c. of the N 0.4 dilution. Following this injection a marked flare-up occurred characterized by redness, local heat, itching and scaling. Eleven grams of scales were removed at one sitting following this flare-up. The dosage was reduced to 0.25 c.c. of the N 0.4 dilution and cautiously increased. After the twenty-fifth injection the lesions presented very few scales and bleeding points, but none of them have disappeared and several new lesions have made their appearance. Whether the injections were in any way responsible for the occurrence of these new lesions is not known. However, no definite therapeutic effects have been obtained.

It is possible that the acute exacerbation of symptoms in this case is comparable to the constitutional reactions occasionally encountered in other fields of allergic therapy. The intradermal skin test is still slightly positive.

CASE 5. White male, aged twenty-seven years. General health good. No family or personal history of allergy. No family history of psoriasis. Typical psoriatic lesions moderately distributed over trunk and extremities. Duration only three weeks. The initial skin test was only weakly positive. The first dose consisted of 0.1 c.c. of the N 0.4 dilution and was increased at three or four day intervals until the sixth dose, which consisted of 0.6 c.c. of the N 0.4 dilution. At this time the skin was smooth and normal except for pigmented areas at the sites of the previous lesions. The intradermal skin test at this time is negative.

CASE 6. White male, aged twenty-six years. General health good. No family or personal history of allergy. No family history of psoriasis. Psoriatic lesions are typical and are extensively distributed over the trunk and extremities; duration eight years. Intradermal skin tests revealed a positive reaction to the N 0.05 dilution. This patient lives in another city and therapeutic injections have not been instigated.

DISCUSSION

Admittedly this small series of cases does not permit definite conclusions to be drawn. However, of the five cases in which desensitization was attempted, only one (Case 4) failed to show at least some improvement. In Case 3, the improvement was slight but definite, while in Cases 1, 3, and 5, apparently satisfactory results occurred. Therapeutic desensitization has not yet been undertaken in Case 6. While it is possible that these results may be explained on the basis of foreign protein therapy, it is felt that they warrant further investigation along the lines of a specific attack upon psoriasis.

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MEDICAL ARTS BLDG.

CLIMACTERIC HYPERSENSITIVENESS TO SUN AND EFFORT

Treatment With Follicular Hormone (Theelin). Preliminary Report of a Case

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AN INCREASING number of cases of hypersensitiveness to cold and to heat, sun and effort are being reported in the literature of allergy. Duke[1, 2] has discussed the subject most thoroughly. The etiology of these responses is as yet obscure. They are variously considered as manifestations of physical allergy, of unusual reactions to food substances[3] or to histamine,[4] or as manifestations of as yet unknown processes.

The following case is of interest in that its predisposing or exciting factor was definitely endocrine and that treatment with a hormone (Theelin, Parke-Davis) produced sudden and almost complete relief. I have been unable to find another case of treatment and relief of physical allergy with follicular hormone in the literature available to me.

CASE REPORT

M. Y., aged forty-three years, white, female, entered the clinic July 5, 1933, complaining of hives and swelling of her hands, lips, eyelids and tongue following exposure to the sun, or after a slight amount of effort. In addition, she complained of faintness, heaviness over the heart, and hot flushes followed by profuse perspiration. The duration of symptoms was approximately eight years.

Family History: One sister has hay fever.

Past History: Negative except for the facts that menstruation began late, at sixteen years, and the menopause began early, at thirty-five years. She has not menstruated for the past eight years.

Present Illness: Sweeping or walking for about ten minutes produces a feeling of heat about the body, whereupon small red spots begin to erupt all over. These grow more numerous and larger, then finally merge until practically the whole body is covered with urticaria. The lips, eyelids, and tongue become swollen, and she feels as if she is unable to swallow. The patient has to lie down for between one and two hours, after which the eruption gradually disappears. Exposure to the direct rays of the sun will produce the urticaria after about

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fifteen minutes. Exposure to heat (hot water in the bathtub, or heat from a fireplace) will not produce the eruption. At times there is a buzzing in the left ear.

There is no cough, dyspnea, or edema of the ankles. She has had no hay fever or asthma. She is not sensitive to any food, as far as she knows. The attacks of hives and swelling have been coming on her only since she began to "change life." Her appetite is good. The bowels are constipated. There is no leucorrhea, and urinary difficulties are absent.

Physical Examination: The upper eyelids are puffed. The skin is pasty in color. There is a pterygium in the right eye. Otherwise the examination is negative except for slight tenderness over McBurney's point.

Pulse 66, temperature 98°, blood pressure 132/110; weight 180 pounds. (The weight has increased from 135 pounds to 180 pounds during the menopause.)

Laboratory Examination: The urine was negative on several examinations. The Wassermann test (blood) was negative. Basal metabolic rate (July 12, 1933) was –30. The complete blood count yielded normal findings.

Course: The patient was given thyroid extract, 3 grains daily; from July 24, 1933, until November 15, 1933. She took this very irregularly without distinct benefit. Once or twice she felt that the tablets might be doing some good, but the final result was one of disappointment. On November 15, 1933, the basal rate was +2.

On September 8, 1933, elimination diet was instituted but this soon proved to be of no benefit and was soon stopped.

On November 15, 1933, she was referred to the allergy clinic, which reported, "This is a case of physical allergy, not sensitive to foods, but sensitive to effort and solar radiations, and we refer this patient to physical therapy for graded desensitization to effort and solar rays."

The patient returned to the medical clinic on April 30, 1934, stating that she had not followed the treatment advised and that she had been to the physical therapy clinic only twice.

At this time it was thought advisable to attempt the use of Theelin experimentally in view of the fact that the symptoms had begun with the menopause and that the menopause was still present as evidenced by hot flushes. She was given 0.5 c.c. on this day.

On May 2, 1934, she stated that she had cleaned two rooms, working about one-half hour, without hives. She was given 1 c.c. of Theelin. On May 4, 1934, the patient stated that she had picked berries in the sun for two hours and had walked a mile without hives. She was given 1 c.c. Theelin. On May 7, 1934, she walked about one mile. A small group of hives broke out on the left arm and disap-

peared in about ten minutes. One c.c. of Theelin was given. On May 9, 1934, the patient stated that she had worked for an hour the previous day, cleaning up several rooms and working harder and longer than she had done in eight years (duration of her menopause). Her ears did not burn, she did not have the feeling of heat from her body, and no hives erupted. The Theelin was omitted on this day. Basal metabolic rate was –17.

On May 11, 1934, the patient had been without Theelin since May 7. On May 10 she had washed her hair. A large patch of hives broke out under both breasts and extended into both axillae. The patient had to lie down for one-half hour until the hives disappeared.

The patient appeared on June 11, 1934, in response to our letter after absenting herself from the clinic. She had had no treatment in the meantime. She said that she had been working and walking and that only a few small hives had been breaking out under the breasts. These few spots appeared only after two hours' effort (as contrasted to fifteen to thirty minutes of effort with widespread eruption prior to the treatment), and had disappeared after ten minutes' rest in a chair (as contrasted to one hour rest in bed).

On four occasions in the past two weeks the patient had sat under the direct rays of the sun for about two hours each time without eruption (as contrasted to fifteen minutes' exposure to the sun with eruption before treatment was started). She appeared tanned from the sun.

On June 10 she had walked fifteen blocks in the hot sun without eruption. She stated, "The past five weeks have been the best in eight years."

DISCUSSION

This case, of course, does not prove that physical allergy, in particular, rests on an endocrinologic basis. It does show, however, that in some cases an endocrine disturbance may be the predisposing or exciting etiologic factor. In this patient, probably, it needed only the constitutional disturbance of the menopause to bring to the surface an underlying hypersensitiveness to effort and to the rays of the sun. The rôle of the thyroid remains undetermined although a definite hypofunction existed.

Brown[5] recommended the use of desiccated whole ovary or corpus luteum "when there are symptoms of ovarion hypofunction such as amenorrhea, irregular or scanty menses or menopause (artificial or natural)." He referred to urticaria in general with ovarian hypofunction and did not mention physical allergy.

Blach[6] reports the case of a fifty-two-year-old woman in whom the climacteric period began in the forty-eighth year. About six months after the menopause began the patient began to be troubled with

urticaria, but not of the physical type. She remained free from symptoms for six months while taking ovarian substance (Ovowop Teichgräber).

Pariser[7] mentioned ovarian substance in urticaria, but referred to urticaria in general and to the probably impotent extracts preceding the present follicular hormones.

SUMMARY

1. A case of physical allergy is reported in which it is considered that the menopause was an etiologic factor.

2. Following the administration of ovarian follicular hormone (Theelin) the patient has received almost complete relief from her symptoms, up to the present time (approximately six weeks).

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ORRIS ROOT FINGERS*

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THAT hypersensitivity to orris root is comparatively common is well known.[2] I should like to call attention to an additional possibility of early contact with orris root, namely, in orris root fingers. These are selected sections of the roots of iris plants which are dried and cut down to a convenient size and shape and are used as teething

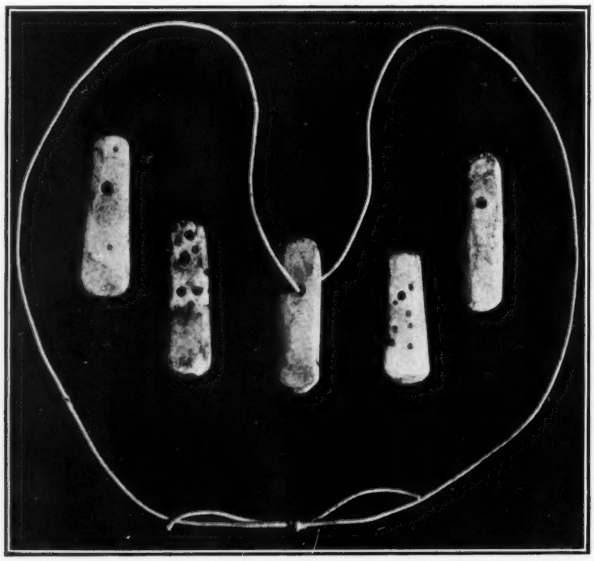

Fig. 1.—Orris root fingers.

sticks for infants. A hole is drilled in one end, through which a cord is slipped to be worn like a necklace about the child's neck (Fig. 1). The sticks have a faint, pleasant scent of violets and a delicate flavor. In consistency they are hard, but not too hard to prevent the child from making slight impressions on the stick with his teeth.

These orris root fingers have been imported into this country from Italy as far back as the older pharmacists can remember.[1] They evidently originated in that country, and it is probable that they have been used by the Italians for many generations. They are also sold extensively in this country, and by no means exclusively to Italians.

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In Rochester, Minnesota, for instance, where there are very few Italian families, there is an occasional request at drug stores for orris root fingers.

The use of orris root fingers, together with the use of other products containing orris root, should, obviously, be discouraged in allergic families.

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Society Proceedings

THE SOCIETY FOR THE STUDY OF ASTHMA AND ALLIED CONDITIONS

TENTH ANNUAL MEETING, ATLANTIC CITY, APRIL 28, 1934

(CONTINUED)

The Effect of Pollen Injections on the Common Cold in Hay Fever Subjects.* LOUIS STERNBERG, M.D., New York, N. Y. (Abstract.)

Patients suffering from seasonal hay fever quite often give a history of frequent attacks of "common cold," that is acute infective rhinitis, especially during the winter months. I have been impressed by clinical observations and the statements of many of these patients that they had lost their susceptibility to these colds during the winter following a course of specific pollen therapy.

This observation is not mentioned in two comprehensive textbooks on allergy, but Cooke found its occurrence in many of his patients.

In this study I was interested only in those cases having no organic nose and throat lesions, such as sinusitis or infected adenoids and tonsils. I also excluded patients suffering from perennial hay fever, from such air-borne substances as dust, dander, orris, etc.

I have reviewed the histories of 200 treated cases of hay fever observed from one to six years. Of these I found that 53 patients gave a previous history of frequent colds. After their first seasonal or perennial course of hay fever treatment with pollen extract, 36 of the 53 stated that they had been relieved of head colds throughout the winter months following the injections. The 17 patients who were not relieved of their winter colds did not get much relief from their hay fever either, so that the relief of these colds appeared to be definitely in proportion to the relief of the hay fever.

The reason for this apparent immunity to the common cold is not known at present. It is presented as a clinical fact that remains to be explained.

DISCUSSION

DR. ALBERT VANDER VEER, New York City.—I have not much to add to what Dr. Sternberg has said. A good many years ago we began to notice that people treated for hay fever told us the next year that they had not had so many winter colds, so now we make it a routine

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question, when they make a report on the hay fever, whether they have been relieved of colds in the winter, and most of them say they do pretty well. By relieving the hay fever we apparently exclude the fall type of sinus infection which predisposes to winter colds. We have a number of patients with clinical late hay fever sensitive to ragweed and also slightly sensitive to grass pollen, but with so little trouble in June they do not require treatment for it. It occurs to me that we might get better results in the ragweed treatment if we treated for the early pollinosis also, even if it is very slight. We shall try that in the future and see whether this will work out; but it is definitely proved that successfully treated late hay fever will protect people against common ordinary colds in the winter. What the explanation is, we do not know.

DR. BANGSTON.—I remember two cases that might give a possible explanation of the problem. One patient had a severe form of hay fever for many years, and it ran five months of each year instead of the usual few weeks. A boy came in who had seasonal asthma which came in October. He was so very sick that even though the ragweed season was over, he was still ill enough to keep him in the clinic. In testing him we found that he gave a marked reaction to his own dust and had to be treated for several weeks with the dust; the following year, instead of having five months of ill health, he had just two days, and required no dust injections, and the period after, the ragweed season, he was completely free.

I wonder whether treating with the main allergen, like ragweed, partial protection may be given to a weaker allergen. It is just an idea but I know that in most of these cases the hay fever was better, too.

DR. J. A. CLARKE, JR., Philadelphia.—I should like to ask whether the patients were tested with other inhalants, for example, house dust, feathers, or animal emanations, as well as the pollen.

DR. LOUIS STERNBERG.—Only those patients who gave a history of frequent colds were tested with all the inhalants; all the others were not.

Department of Reviews and Abstracts

Selected Abstracts

Intramucous Autoserotherapy in Asthma. Jacquelin, A., and Bonnett, G.: Presse méd. **42**: 249, 1934.

This work is a continuation of one previously reported by the authors. It is based on the success of treatment of certain cases of allergic skin disorders as eczema and urticaria by autoserotherapy, wherein serum is injected into the skin. On the other hand, it was noted that this treatment gave poor results in bronchial and nasal manifestations of allergy. This led to the thought that the latter cases should be treated by injections into the mucous membranes, and this thought was rationalized by immunologic considerations.

The method consists in drawing 10 c.c. of blood from a vein, and separating serum from the clot. The serum is injected into the nasal mucous membrane through an intradermal testing needle. The site of injection is in the mucous membrane over the inferior turbinate. A bleb in the mucosa must appear if the injection is correctly made. It is not necessary to sterilize the site of injection. The procedure is not painful and has been carried out in infants. One injects 0.2 c.c., then 0.3 c.c., 0.4 c.c., and 0.5 c.c. of serum daily for 20 doses

In this series and another previously reported, 51 cases of asthma and vasomotor rhinitis were thus treated. In the majority of cases the results were good, at times with complete amelioration of symptoms. Case reports are given. It is concluded that this form of therapy deserves a prominent place in the treatment of asthma and allied conditions. Since the treatment is, however, more complicated than usual, requiring, as it does, daily injections, it may be reserved for the more severe cases which it frequently ameliorates.

Gastrointestinal Allergy Associated With Transient Intraventricular Block. Healy, J. C., Gallison, D. T., and Brundo, J.: New England J. Med. **210**: 123, 1934.

A case report is given of a forty-six-year-old man with a negative past history, and a present illness of three weeks' duration. The onset was of severe upper abdominal pain, nausea, and vomiting for two days.

This was followed by diarrhea and lower abdominal pain. Physical examination showed the heart to be questionably enlarged to the left, and a distended abdomen.

On laboratory examination the significant findings showed slight enlargement of the heart to the left by x-ray examination; a gastrointestinal x-ray series showed increased haustral markings sufficient to warrant a diagnosis of spastic colitis; an electrocardiogram showed slurring of the QRS complexes in Leads I and II and notching in Lead III, a diphasic T-wave in Lead II, and slight left axis deviation; the stools were found to contain many eosinophilic leucocytes. A diagnosis of spastic colitis was made.

The patient improved, and then had a recurrence of symptoms. At this time the white blood cells were 34,000, of which 70 per cent were eosinophiles. Precipitin tests for trichiniasis were reported to be positive, but biopsy examination of the deltoid muscle showed no evidence of infestation. Because of the eosinophilia, scratch tests were done, and various derivatives of wheat gave positive reactions. On a wheat-free diet, symptoms improved markedly. During this time there was recurrence after deliberate taking of wheat, and again improvement when off of wheat.

Striking changes in the electrocardiogram occurred as the patient improved. The electrocardiograms taken before the patient's wheat sensitivity was discovered revealed definite conduction defect and a suggestion of coronary disease. After wheat had been eliminated from the diet, there was marked restoration toward normal, excepting the diphasic T-wave and left axis deviation. After the patient had broken his diet, the electrocardiographic changes reverted to the original, and again disappeared as his symptoms improved.

Following the suggestion of Richet and Coudier, the patient was given two teaspoonfuls of mineral oil before each meal for the allergic intestinal condition, and this treatment seemed quite effective.

The literature dealing with the possible allergic nature of cardiac conditions is given.

Angina Pectoris and Allergy. Von Eiselberg, K. P.: Klin. Wchnschr. **13**: 619, 1934.

By clinical observations as well as by animal experimentation it appears that angina pectoris with definite electrocardiographic changes may be induced by anaphylaxis. Two patients with food allergy are reported. In the first, carrots and tomatoes were found to be responsible for symptoms. In the second, milk, egg, and other allergens were the offending foods. The first patient was relieved by an elimination diet. The second one was desensitized with propeptan according to

the Luithlen-Urbach method. Other causes for symptoms were ruled out. A considerable bibliography dealing with various phases of the subject is appended.

"Isalon," a New Ephedrine Derivative, in the Treatment of Bronchial Asthma. Handovsky, H.: München. med. Wchnschr. **81**: 326, 1934.

"Isalon," an ephedrine derivative, was tested both clinically and in animals for its effect on the bronchi and was found to be strongly antibronchospastic. This was tested by perfusing lungs under experimental conditions with Tyrodes' solution containing dilutions of pilocarpin. After the injection of Isalon much greater concentrations of pilocarpin were required to cause bronchial constriction than at the beginning of the experiment. At the same time, it has but little or practically no effect on the blood pressure. Dogs narcotized with chloralose, showed after Isalon a fall in blood pressure, whereas the same dose of ephedrine under similar conditions showed a rise in pressure. In man, therapeutic doses have little effect. Gram for gram, it is only half as toxic as ephedrine. Unlike adrenalin, it is not quickly destroyed. Its only effect is on the sympathetic endings.

"Isalon," a Synthetic Derivative of Ephedrine, as a Specific Asthma Remedy. Kubeja, E.: München. med. Wchnschr. **81**: 327, 1934.

Clinical observations on asthmatic patients treated with "Isalon" show that it has a very favorable effect in controlling paroxysms. A particular virtue is that its action is essentially on the bronchi, and it has practically no effect on the vasculature. The various side effects induced by adrenalin and ephedrine, such as headache, restlessness, elevation of blood pressure, etc., are scarcely experienced by the use of Isalon. Morever, the drug has a more prolonged effect.

Ephedrine and Pseudo-Ephedrine in Asthma, Bronchial Asthma and Enuresis. Christopherson, J. B., and Broadbent, M.: Brit. M. J. **1**: 978, 1934.

The studies undertaken were part of an inquiry arranged by the Therapeutic Trials Committee of the Medical Research Council into the relative values of ephedrine and pseudo-ephedrine. The two alkaloids occur together in varying proportions in several species of *Ephedra*. They have the same chemical structure but differ physically.

Patients with asthma were divided into (a) spasmodic cases, that is, those without evidence of bronchitis, and (b) bronchial cases with gross evidence of bronchitis. In spasmodic asthma 24 patients were treated successively with the two alkaloids, there being an interval between the courses of the two drugs during which the patients were treated with inert tablets. The drugs were given ordinarily at bed-

time in doses of ¼ to 2 grains, according to the age of the patient. Five patients had no bad attacks during the period of observation; 15 patients were relieved of wheezing by both alkaloids, but pseudo-ephedrine was less effective.

The results in 37 cases of so-called bronchial asthma were as follows: 20 patients had no severe attacks, and in 15 of these pseudo-ephedrine taken regularly relieved the mild constant wheezing. In 9 it was effective in relieving slight attacks. In the 8 cases in which acute attacks occurred, it was not effective.

In comparing further the action of the two alkaloids, it was concluded that pseudo-ephedrine is valuable as a mild bronchial sympathetic stimulant, sufficiently active to give relief in the chronic wheezing and dyspnea of asthma. It is a useful prophylactic against nocturnal dyspnea of asthma, and against the dyspnea of exertion in chronic bronchitis. It does not effectively take the place of ephedrine, still less of adrenalin, in acute attacks. It is noncumulative in its action, and produces fewer side effects than ephedrine in the same dosage. Generally speaking, although not always, about double the dosage of pseudo-ephedrine is required to produce the equivalent physiologic effect of a given dose of ephedrine.

Book Review

ALLERGISCHE DIATHESE UND ALLERGISCHE ERKRANKUNGEN, BY HUGO KÄMMERER. Ed. 2, Munich, 1934, 359 pages, J. F. Bergmann.

This book is an important contribution to allergy in that it is the most comprehensive review of the subject written in the German language. The first edition was published in 1926, and the present volume is greatly enlarged to cover the great amount of work undertaken in the intervening years. The book is divided into two parts. The first, or general, section deals with a generalized exposition of allergy and experimental anaphylaxis. There is a very interesting chapter on the allergic constitution which includes hereditary influences, psychoneurotic make-up, the changes in old age, vegetative nervous system response, body configuration, rôle of internal secretions, and other factors which contribute to the allergic "diathesis." The first section deals also at considerable length with idiosyncrasies, diagnostic methods, and therapeutic measures. The second section is devoted to specific clinical disorders including both these known and those suspected to be of allergic origin. Among other symptom-complexes are included eosinophilic catarrh, epilepsy, arthritis, and affections of the kidneys and cardiovascular system.

The bibliography is extensive and general. The work is encyclopedic in its scope, as it includes both the experimental and the clinical data, even those in which there is debate as to whether or not they belong to allergy. In such instances, the material is presented fairly, and the author frequently offers his own opinion. As a reference book this work fills a place in that it elaborates upon much not to be found in other reviews.